CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020884

PHARMACOLOGY REVIEW(S)

NOV - 9 1999

PHARMACOLOGIST'S REVIEW OF NDA 20,884
(Amendment dated November 4, 1999)

Sponsor & Address: Boehringer Ingelheim Pharmaceuticals, Inc.
Ridgefield, CT

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Date of Submission: November 4, 1999

Date of HFD-180 Receipt: November 5, 1999

Date of Review: November 9, 1999

DRUG: Aggrenox (dipyridamole 200 mg / aspirin 25 mg), Capsules

CATEGORY: Anti-platelet agents.

Submission Contents: Revised label.

In this submission, sponsor submitted revised label in response to our recommendations in the pharmacology review dated on September 22, 1999. In this review, sponsor was recommended to remove the following information from the final label: "Combination of Dipyridamole and Aspirin: In a 105 week oral (in feed) study in mice and in a 125 week oral (in feed) study in rats, combination of dipyridamole and aspirin in a ratio of 1:5 produced no significant carcinogenic effects at doses of 50, 150, and 450 mg/kg/day. The dose of aspirin at 375 mg/kg/day (1125 mg/m^2 in mice or 2250 mg/m^2/day in rats) represents ~30 or 61 times the recommended human dose of aspirin in AGGRENOX (1 mg/kg/day or 37 mg/m^2/day) on a body surface area basis."

The suggested version for the final label is as follows:

Sponsor was also suggested to include the following under Overdose section in the final label as recommended previously:

[redacted]

SUMMARY AND EVALUATION:

Sponsor submitted revised label in response to our recommendations in the pharmacology review dated on September 22, 1999. These recommendations were conveyed to sponsor on October 4, 1999 via fax. Sponsor has revised the final label as recommended.

RECOMMENDATION: None.

APPEARS THIS WAY
ON ORIGINAL

/S/ 11/9/99

Ke Zhang, Ph.D.

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang

/S/ 11/9/99

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22 1999

PHARMACOLOGIST'S REVIEW OF NDA 20,884
(Amendment dated August 6, 1999)

Sponsor & Address: Boehringer Ingelheim Pharmaceuticals, Inc.
Ridgefield, CT

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Date of Submission: August 6, 1999

Date of HFD-180 Receipt: August 9, 1999

Date of Review: September 20, 1999

DRUG: Aggrenox (dipyridamole 200 mg / aspirin 25 mg), Capsules

CATEGORY: Anti-platelet agents.

Submission Contents: Revised label.

In this submission, sponsor submitted revised label in response to our approvable letter dated June 15, 1999. Sponsor has incorporated the recommendations on Carcinogenesis, Mutagenesis, Impairment of Fertility, and Pregnancy into the final label but did not revise the label for Overdose section. Sponsor believed that the value of the overdose section is limited since there are considerable clinical experience with aspirin and dipyridamole components of Aggrenox. Aggrenox contains 200 mg dipyridamole and 25 mg aspirin in a ratio of dipyridamole to aspirin of 8:1. No toxicity studies were conducted using drug combination of aspirin and dipyridamole in a ratio of dipyridamole to aspirin of 8:1 except this single dose acute oral toxicity study in rats. Therefore, we recommend sponsor to include the information under Overdose section in the final label as recommended previously.

Based on the new information submitted to this NDA on July 12 and August 20, 1999, we suggest sponsor to remove the following information from the final label: "Combination of Dipyridamole and Aspirin: In a 105 week oral (in feed) study in mice and in a 125 week oral (in feed) study in rats, combination of dipyridamole and aspirin in a ratio of 1:5 produced no significant carcinogenic effects at doses of 50, 150, and 450 mg/kg/day. The dose of aspirin at 375 mg/kg/day (1125 mg/m^2 in mice or 2250 mg/m^2/day in rats) represents ~30 or 61 times the recommended human dose of aspirin in AGGRENOX (1 mg/kg/day or 37 mg/m^2/day) on a body surface

area basis." This was based on the following facts: (1) that Aggrenox contains 200 mg dipyridamole and 25 mg aspirin [redacted] (2) that the carcinogenicity studies were conducted using the drug combination of dipyridamole and aspirin in a ratio of 1:5, (3) that there were significant deviations of conduct of these carcinogenicity studies from GLP regulations, (4) that the 2-year carcinogenicity studies with Aggrenox in animals are not needed since persantine (dipyridamole) itself was negative in the 111-week oral carcinogenicity study in mice and 128-142 week oral carcinogenicity study in rats and aspirin has been widely used in humans for many years and there was no indication of tumorigenicity, and (5) there is no pharmacokinetic interaction between the drug substances in humans.

Following is the sponsor's original version:

[redacted]

The suggested version for the final label is as follows:

[redacted]

APPEARS THIS WAY
ON ORIGINAL

SUMMARY AND EVALUATION:

Aggrenox contains 200 mg dipyridamole and 25 mg aspirin in a [redacted] The NDA of Aggrenox (20,884) is approvable [redacted]

Sponsor has incorporated the recommendations on Carcinogenesis, Mutagenesis, Impairment of Fertility, and Pregnancy into the final label but did not revise the label for Overdose section as recommended. Sponsor believed that the value of the overdose section is limited since there are considerable clinical experience with aspirin and dipyridamole components of Aggrenox. The single dose acute oral toxicity study in rats was the only toxicity study conducted using drug combination of aspirin and dipyridamole [redacted] Therefore, we recommend sponsor revise the label (Overdose section) as recommended previously.

No carcinogenicity studies were conducted with drug combination of dipyridamole and aspirin in a ratio of 8:1 as in Aggrenox. The 105-week oral (in feed) carcinogenicity study in mice and 125-week oral (in feed) carcinogenicity study in rats were conducted using the drug combination of dipyridamole and aspirin in a ratio of 1:5. There were significant deviations of conduct of these carcinogenicity studies from GLP regulations. Persantine (dipyridamole) itself was negative in the 111-week oral carcinogenicity study in mice and 128-142 week oral carcinogenicity study in rats. Aspirin has been widely used in humans for many years and there was no indication of tumorigenicity. There is no pharmacokinetic interaction between the drug substances in humans. Therefore, the 2-year carcinogenicity studies in animals with Aggrenox are not needed and the results of the 105-week oral (in feed) carcinogenicity study in mice and 125-week oral (in feed) carcinogenicity study in rats conducted using the drug combination of dipyridamole and aspirin in a ratio of 1:5 do not provide any useful information in the label and should be removed from the final label.

RECOMMENDATION:

The recommended changes in the final label should be implemented.

/S/ 9/20/99
Ke Zhang, Ph.D.

/S/ 9/22/99

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang

R/D Init.: J. Choudary 9/17/99

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APPEARS THIS WAY
ON ORIGINAL

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PHARMACOLOGIST'S REVIEW OF NDA 20,884
(Amendments dated July 12 and August 20, 1999)

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

Sponsor & Address: Boehringer Ingelheim Pharmaceuticals, Inc.
Ridgefield, CT

Date of HFD-180 Receipt: July 14 and August 23, 1999

Date of Review: September 8, 1999

DRUG: Aggrenox (dipyridamole 200 mg / aspirin 25 mg), Capsules

CATEGORY: Anti-platelet agents.

Submission Contents: Response to Executive CAC recommendations dated May 11, 1999.

BACKGROUND: In this NDA (20,884), sponsor submitted a 105-week oral (in feed) carcinogenicity study in mice and a 125-week oral (in feed) carcinogenicity study in rats. These studies were discussed by The Executive Carcinogenicity Assessment Committee (Ex. CAC) on May 11, 1999. Following recommendations were made by the committee: **(1)** to clarify how the conduct of the mouse and rat studies deviated from GLP regulations and the significance of these deviations, **(2)** to have the statisticians do a test to determine the significance of the incidence of the thymoma in the rat study, and **(3)** to obtain available data on aspirin plasma clearance in mice versus humans from literature. These recommendations were conveyed to sponsor in the Division's letter on June 4, 1999. Sponsor submitted their responses to the recommendations in this submission.

1. Clarify how the conduct of the mouse and rat studies deviated from GLP regulations and the significance of these studies.

These studies were conducted by the [redacted]. The deviations from GLP regulations were summarized by [redacted] in Attachment 1 on pages 43 and 44.

In brief, studies were not inspected or examined by an independent Quality Assurance Unit (QAU) and the reports did not include any statement on GLP compliance, there were no Standard Operating Procedure (SOP), and most importantly, the dietary admixtures were not analyzed for drug concentrations. Therefore, the drug combinations consumed by the animals were not known. Particularly, in mice no treatment related changes were seen at all doses and it was not clear whether MTD was reached at the doses tested. In conclusion, these studies are considered invalid due to these deviations from GLP regulations.

2. Perform a test to determine the significance of the incidence of the thymoma in the rat study.

Sponsor conducted a statistical analysis on the incidence of thymoma in the rat study using Fisher's exact test and the result was summarized in a table on page 3. This table is attached below.

DPD + ASA: INCIDENCE OF THYMOMA IN RATS AFTER 125 WEEKS

Dose groups	Control		50 mg/kg		150 mg/kg		450 mg/kg	
Sex	M	F	M	F	M	F	M	F
Number of animals	100	100	50	50	50	50	50	50
Number of thymoma	1	2	4	4	4	7	2	3
% thymoma	1	2	8	8	8	14	4	6
P % (Fisher exact)	-	-	4.3*	9.6	4.3*	0.7**	25.8	20.7

The incidence of thymoma was significantly different from controls in males of the low and mid dose groups at 5% level (*) and in females of the mid dose at the 1% level (**). No increase was present in high dose animals of either sex. Since there was no consistent dose-relationship in the incidence, sponsor did not perform a trend test.

Dr. Karl Lin (CDER statistician) has also conducted mortality adjusted exact permutation trend and pairwise tests on this tumor incidence and concluded that there was no statistical significance for both males and females in both pairwise and trend tests (see Memo to this NDA dated May 21, 1999).

Sponsor was unable to provide historical control data on the rat strain used in the testing laboratory but included published historical control data for CHbb:THOM(SPF) rats by

Sponsor stated that thymoma is a frequent tumor in this strain rat (up to 20%). However, in the published historical data provided in this submission, the tumor rate in the thymus was up to 20% but tumor type was not specified.

3. Submit available data on aspirin plasma clearance in mice versus humans from literature.

The data on aspirin (ASA) plasma clearance in mice and humans were summarized in Table 3:1 on pages 6 and 7. These tables are attached below (SA = Salicylate).

Table 3: 1 Clearance data of aspirin in humans (and mice)

Ref. doc. Document	remark	analyte	dose mg	dose mg/kg	AUC µMol*h/ml	AUC µg*h/ml	CL ml/(min*kg)	CL ml/min	CL l/h	CL l/h*kg
M1	Mice	SA		65		351.8				0.185
M2	Mice	SA		200		851				0.24
U98-2379		SA	25	0.33		4.00			6.25	0.08
U98-2379		ASA	25	0.33		0.20			125.00	1.67
H1		SA	325	4.33	539	67.91			4.79	0.06
H2	min value	ASA	1000	13.33		17.50	12.7		57.15	0.76
H2	max value	ASA	1000	13.33		7.80	28.5		128.25	1.71
H3	generic asp	ASA	500	6.67		5.40			92.59	1.23
H3	Aspirin	ASA	500	6.67		5.03			99.40	1.33
H4		ASA				0.00		650	39.00	0.52
H5		SA	1300	17.33		373.40			3.48	0.05
H6	eld-woman	SA	650			300.93	0.48		2.16	0.03
H6	young-woman	SA	650			232.97	0.62		2.79	0.04

Table 3: 1 Clearance data of aspirin in humans (and mice)

Ref. doc. Document	remark	analyte	dose mg	Dose Mg/kg	AUC µMol*h/ml	AUC µg*h/ml	CL ml/(min*kg)	CL ml/min	CL l/h	CL l/h*kg
H6	eld-woman	SA	650			236.79	0.61		2.75	0.04
H6	young-woman	SA	650			218.86	0.66		2.97	0.04
H6	overweight	SA	650			283.22	0.51		2.30	0.03
H6	norm-weight	SA	650			209.34	0.69		3.11	0.04
H7		ASA	975			13.00			75.00	1.00
H7		SA	975			395.00			2.47	0.03
H8		SA	650.			283.00			2.30	0.03
H9		ASA	1000.		60.50	7.62			131.18	1.75
H9		SA	1000.		2867.	361.24			2.77	0.04
H10	women	SA	1000.					20.02	1.20	0.02
H10	man	SA	1000.					38.35	2.30	0.03
H11			1140.						1.65	0.022
H12		SA	975 q.i.d.						0.71	0.01
H13			975 q.i.d.			916.00			1.06	0.01
H14	ewe-man	ASA						764	45.84	0.61
H15	admin of SA	SA	10 mg/kg						2.06	0.03

SUMMARY AND EVALUATION:

Aggrenox contains 200 mg dipyridamole and 25 mg aspirin in a ratio of dipyridamole to aspirin of 8:1. The NDA of Aggrenox (20,884) is approvable.

No carcinogenicity studies were conducted with drug combination of dipyridamole and aspirin in a ratio of 8:1 as in Aggrenox. However, there were a 105-week oral (in feed) carcinogenicity study in mice and a 125-week oral (in feed) carcinogenicity study in rats conducted using the drug combination of dipyridamole and aspirin in a ratio of 1:5. These studies were reviewed on April 30, 1999 (Pharmacology review) and on May 11, 1999 (Executive CAC). The Executive CAC recommended sponsor to clarify how the conduct of the mouse and rat studies deviated from GLP regulations and the significance of these deviations, to have the statisticians do a test to determine the significance of the incidence of the thymoma in the rat study, and

to obtain available data on aspirin plasma clearance in mice versus humans from literature. These recommendations were conveyed to sponsor in the Division's letter on June 4, 1999. Sponsor submitted their responses to the recommendations in this submission.

There were significant deviations from GLP regulations in these studies including lack of standard operating procedures, analysis of dietary admixtures, and inspection and examination by an independent Quality Assurance Unit. It should be emphasized that these studies were conducted using the drug combination of dipyridamole and aspirin in a ratio of 1:5 and no carcinogenicity studies were conducted with drug combination of dipyridamole and aspirin in a ratio of ~~8:1 as in~~ Aggrenox. In addition, it was concluded in the Pharmacology review dated April 30, 1999 that the 2-year carcinogenicity studies in animals are not needed since persantine (dipyridamole) itself was negative in the 111-week oral carcinogenicity study in mice and 128-142 week oral carcinogenicity study in rats and aspirin has been widely used in humans for many years and there was no indication of tumorigenicity. There is no pharmacokinetic interaction between the drug substances in humans. Therefore, the information of the carcinogenicity studies in mice and rats with drug combination of dipyridamole and aspirin in a ratio of 1:5 should not be included in the labeling.

Sponsor conducted a statistical analysis on the incidence of thymoma in the rat study using Fisher's exact test. The results indicate that the incidence of thymoma was significantly different from controls in males of the low and mid dose groups at 5% level (*) and in females of the mid dose at the 1% level (**). Nc increase was present in high dose animals of either sex. Sponsor did not perform a trend test due to lack of consistent dose-relationship in the incidence. The mortality adjusted exact permutation trend and pairwise tests on this tumor incidence were performed by CDER statistician, Dr. Karl Lin. The results indicated that there was no statistical significance for both males and females in both pairwise and trend tests.

The plasma clearance of aspirin or salicylates was 0.185 - 0.24 l/h·kg in mice and 0.01-1.75 l/h·kg in humans.

RECOMMENDATION:

Based on the facts (1) that these carcinogenicity studies were conducted using the drug combination of dipyridamole and aspirin in a ratio of 1:5, (2) that there were significant deviations of conduct of these carcinogenicity studies from GLP regulations, and (3) that the 2-year carcinogenicity studies in animals are not needed, the information of the carcinogenicity studies in mice and rats with drug combination of dipyridamole and aspirin in a ratio of 1:5 should not be included in the labeling. Sponsor should be asked to remove this information from the final label for Aggrenox.

/S/ 9/8/99
Ke Zhang, Ph.D.

APPEARS THIS WAY
ON ORIGINAL

/S/ 9/14/99

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang

R/D Init.: J. Choudary 8/22/99

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NDA 20,884 **REVIEW # 1**

Sponsor & Address: Boehringer Ingelheim
Ridgefield, CT

Reviewer: Ke Zhang, Ph.D.
Pharmacologist

APR 30 1999

Date of Submission: December 15, 1998

Date of HFD-180 Receipt: December 17, 1998

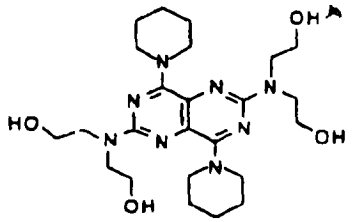
Date of Review: April 30, 1999

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Original Summary

DRUG: AGGRENOX (dipyridamole 200 mg/aspirin 25 mg), Capsules

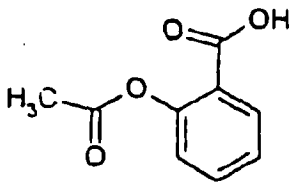
Dipyridamole (persantine):

2,6-bis(diethanolamino)-4,8-dipiperidino-pyimido(5,4-d)pyrimidine



$C_{24}H_{40}N_8O_4$ MW: 504.63

Aspirin (acetylsalicylic acid): Benzoic acid,2-(acetyloxy)-



$C_9H_8O_4$ MW: 180.16

CATEGORY: Antiplatelet agents

Related NDA: NDA 12,836

Marketing Indications and Dose:

The recommended oral dose is one capsule twice daily. One capsule contains 200 mg dipyridamole and 25 mg aspirin.

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of Study	Study #	Lot #	Lab	Page #
Pharmacology				3-5
Absorption, Distribution, Metabolism and Excretion (ADME):				6-8
Bioavailability and pharmacokinetics of persantine in dogs	084-0706	---		
Acute Toxicity Study				8-9
Acute oral/i.p. toxicity study in mice and rats	E78-738	---	1	
Acute oral toxicity study in mice	077-0236	---	2	
Acute oral toxicity study in rats	96B031	740044	2	
Acute oral toxicity study in rats	071-0168	151471 ---	2	
Acute oral toxicity study in dogs	071-0167	FN 2939, WE 0086	2	
Subacute to Subchronic Toxicity Study				
3-month oral toxicity study in rats	073-0185	Z 7391	2	10-11
24-week oral toxicity study in rats	075-0196	178/02	1	11-12
24-week oral toxicity study in dogs	075-0196	CA 73 173 II	1	12-14
27-week oral toxicity in dogs	078-0202	CA 0257 II FN 5262, WE 1360	2	14-16
Carcinogenicity Study				
101-week oral toxicity study in mice	079-0257	50621, WE 0492	3	17-23
125-week oral toxicity study in rats	079-0258	50621, WE 0492	3	24-31
Reproductive Toxicity Study				
Segment II oral teratology study in rats	078-0203	50215a	4	31-33
Segment II oral teratology study in rabbits	078-0204	50215a	4	33-35
Mutagenicity				
Ames test	078-0206	FN 4682, 155266	2	36-37
In vivo chromosome aberration test in mice and hamsters	078-0201	---	2	37
Oral micronucleus test in mice and hamsters	077-0240	---	2	38
Dominant lethal test in mice	077-0237	---	2	38-41

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PHARMACOLOGY:

Primary Activity

Both dipyridamole and aspirin have been used in humans for many years and extensive information about their pharmacological properties has been accumulated over the years. Following are the brief summaries of pharmacology of each compound.

Dipyridamole:

Dipyridamole is an antiplatelet agent. Its antiplatelet activity is related to its inhibition of adenosine uptake, inhibition of cGMP phosphodiesterase ~~or increase~~ in prostacyclin (PGI_2) level. An *in vitro* study demonstrated that dipyridamole inhibits adenosine uptake into the red blood cells in the whole blood by 90% at concentration of 1 μM. The *ex vivo* studies indicated that pretreatment of healthy volunteers with dipyridamole at a daily dose of 400 mg for 3 or 4 days inhibits adenosine uptake by 92% or increases adenosine level by 60% in the whole blood. Adenosine then activates the platelet A_2-receptor, and stimulates platelet adenylate cyclase which in turn increases cAMP level and inhibits platelet activation. The *in vitro* studies also demonstrated that dipyridamole inhibits cGMP phosphodiesterase in isolated human platelet, rabbit aorta, and rod cells with IC_{50} of 1.6, 7 and 0.4 μM, respectively. By inhibiting cGMP phosphodiesterase which inactivates cGMP, dipyridamole increases the cGMP level in the platelet, and like cAMP, cGMP can inhibit platelet aggregation. Dipyridamole increases the release of PGI_2 by ~13% (1 μM) and 49% (10 μM) from rat aorta and by 79% (5 μM) and 142% (10 μM) in human veins. Pretreatment of healthy volunteers with dipyridamole at oral dose of 375 mg/day for 7 days increases plasma level of PGI_2 by 40%. PGI_2 also activate platelet adenylate cyclase which in turn increases cAMP level and inhibit platelet activation.

Aspirin:

It is known that ASA irreversibly inhibits platelet cyclooxygenase and reduces the production of thromboxane A_2 (TXA_2). The latter is a potent platelet aggregator. As a result, aspirin markedly inhibits platelet aggregation.

Dipyridamole and Aspirin:

Since dipyridamole and aspirin inhibit platelet aggregation via independent mechanisms, use of both drugs in combination is expected to produce an additive antiplatelet effect. This was demonstrated in a number of animal models in rats, rabbits and dogs.

In an electrical-induced venous thrombus model in rats, treatment with combination of dipyridamole and ASA at doses of 5/0.05 mg/kg (100:1), 2/0.05 mg/kg (40:1) and 5/5 mg/kg (1:1) reduced the size of thrombus by ~30%, 50% and 38%, respectively. In a rabbit model, the thrombus formation was induced by stenosing the aorta and damaging the endothelium. In this model, treatment with either dipyridamole (i.v. dose of 5 mg/kg) or ASA (i.v. doses up to 1 mg/kg) alone failed to inhibit the thrombus formation. However, combination of dipyridamole and ASA significantly inhibits the thrombus formation and improves the blood flow by ~80% (5/0.1 mg/kg, 50:1), 52% (5/0.05 mg/kg, 100:1) and 23% (5/0.5 mg/kg, 10:1). Effects of combination of dipyridamole and aspirin on platelet accumulation were studied in an arterial graft in dogs. The results indicated that oral administration of combination of dipyridamole (5 mg/kg/day) and aspirin (2 mg/kg/day) for 3 days significantly reduced platelet accumulation within the graft by ~75%.

Secondary Activity

1. Cardiovascular System: Oral administration of combination (8:1) of dipyridamole and ASA at 50/6.25, 100/12.5 and 200/25 mg/kg/day for 7 days had no effects on arterial blood pressure in conscious rats. In a study in anesthetized pigs, intravenous infusion of combination (8:1) of dipyridamole and ASA at doses up to 10/1.25 mg/kg over 20 minutes had no significant effects on the heart rate, left ventricular dp/dt_{max}, PQ and QT intervals and cardiac output. However, the systolic and diastolic arterial pressure, maximum left ventricular pressure and total peripheral resistance were dose dependently decreased and the results were summarized in a table on page 72 in volume 19. This table is attached below.

Effects of dipyridamole + ASA in anesthetized pigs
(n = 5, % change from pretreatment control at the end of each infusion)

Dose dipyridamole + ASA (mg/kg i.v.)	Systolic arterial pressure	Diastolic arterial pressure	Maximal left ventricular pressure	Total peripheral resistance
0.01 + 0.00125	- 0.9	- 3.0	- 0.5	+ 0.4
0.03 + 0.00375	- 5.3	- 10.1	- 3.5	- 3.5
0.1 + 0.0125	- 12.3	- 19.5	- 13.1	- 10.9
0.3 + 0.375	- 23.6	- 32.4	- 22.3	- 21.0
1.0 + 0.125	- 34.1	- 44.2	- 27.5	- 32.7
3.0 + 0.375	- 41.1	- 51.7	- 31.7	- 42.8
10.0 + 1.25	- 45.0	- 58.8	- 33.6	- 54.5

2. Gastrointestinal System: Oral administration of combination (8:1) of dipyridamole and ASA at 50/6.25, 100/12.5 and 200/25 mg/kg/day for 7 days had no irritable effects on rat gastric mucosa.

3. Renal System: A single oral dose of combination of dipyridamole and ASA at 60/7.5 mg/kg did not affect urine volume, micro-protein, creatinine, glucose, Na^+, Cl^- and K^+ levels in dogs.

In summary, dipyridamole and aspirin are antiplatelet agents and inhibit platelet aggregation via independent mechanisms. For example, the antiplatelet activity of dipyridamole is believed to be related to its inhibition of adenosine uptake, inhibition of cGMP phosphodiesterase or increase in prostacyclin (PGI_2) level. By inhibition of adenosine uptake, it increases plasma level of adenosine which would activate the platelet A_2-receptor, stimulate platelet adenylate cyclase, and elevate platelet cAMP level. PGI_2 also activate platelet adenylate cyclase and increases cAMP level. Inhibition of cGMP phosphodiesterease would increases cGMP level in the platelet. Both cAMP and cGMP can inhibit platelet aggregation. On the other hand, ASA irreversibly inhibits platelet cyclooxygenase and reduces the production of thromboxane A_2 (TXA_2). TXA_2 is a potent platelet aggregator. Therefore, use of both drugs in combination would produce an additive antiplatelet effect.

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME):

Both dipyridamole and aspirin have been used in humans for many years and extensive information about their pharmacokinetic properties has been accumulated over the years. Following are the brief summaries of pharmacokinetics of each compound.

Dipyridamole:

Dipyridamole is quickly absorbed following oral administration and the oral bioavailability is up to 77% in rats, 68% in dogs, and 70% in humans. After oral dose of 10 mg/kg dipyridamole in rats, the peak plasma level of dipyridamole (0.1-0.25 µg/ml) is reached within 20 minutes. After oral dose of 50 mg dipyridamole in dogs, the peak plasma level of dipyridamole (~1.2 µg/ml) is obtained at ~1.2 hours. The half life is ~10 hours in both rats and dogs. The pharmacokinetic properties of dipyridamole in dogs and humans were presented in Table 5.1.3.1:2 on page 30 in volume 19 and this table is attached below.

TABLE 5.1.3.1: 2 Comparison of pharmacokinetic parameters in dog and man

Species	dog		man	
	intravenous	oral	intravenous	oral
Dose mg/subject	25/5 minutes	50	60/1.25 hours	200 ER b.i.d.
Dose mg/kg	2	4	0.8	5.3
AUC [µg*h/ml]	1.77	1.18	4.30	7.87
C_{max} [µg/ml]	1.94	0.602	1.77	1.42
$t_{½}$ [h]	10	10	14.8	11.3

Sources: dog data are derived from U84-0706, those for man from U96-2190

Dipyridamole is highly bound to protein (~91% in mice, 92% in rats, 86% in guinea pigs, 96% in rabbits, 88% in dogs, 89% in pigs and 91% in humans). In rats, the major metabolite identified is dipyridamole-monoglucuronide accounted for 87% of the dose given. Dipyridamole-glucuronide is also identified in dogs and humans. Following oral administration of ^{14}C-dipyridamole in rats, the radioactivity is recovered mainly in the feces (76% in rats) and only ~6% in the urine within 72 hours. In guinea pigs, ~70% of the oral dose of dipyridamole (25 or 50 mg) is recovered in feces. Following i.v. administration of dipyridamole in mice and rabbits, approximately 80% of the dose administered is excreted into the intestine within 5 hours, suggesting that the biliary excretion is the major excretion pathway in these species.

Aspirin:

Following oral administration, ASA is quickly and usually completely absorbed from gastrointestinal tract. The peak plasma levels of ASA vary between 15 minutes for soluble tablet to 360 minutes for extended-release formulation. ASA is quickly metabolized to salicylic acid with a half life of 13-20 minutes. Salicylic acid is highly bound to protein (80-90%) and has an apparent volume distribution of 9.6-12.7 l in adults. Salicylic acid declines relatively slower with a half life of 2-3 hours.

Interaction of Dipyridamole and Aspirin:

Aggrenox contains 200 mg dipyridamole and 25 mg aspirin (8:1). There are no pharmacokinetic studies of interaction of dipyridamole and aspirin in a ratio of 8:1 (dipyridamole:aspirin) in animals. However, a pharmacokinetic study in dogs, dipyridamole was given to dogs by an i.v. dose at 25 mg and oral dose of 50 mg. Different formulations of dipyridamole were used in this study including capsule with fumaric acid, tablet without acid, and tablet with orange juice. The oral bioavailability of dipyridamole (50 mg) was 68.1, 32.9 and 56.4% for the formulations of capsule with fumaric acid, tablet without acid, and tablet with orange juice, respectively. In contrast, when dipyridamole (tablet without acid) was given to dogs with combination of aspirin (325 mg), the oral bioavailability of dipyridamole was 55.7% which was much higher than dipyridamole alone (32.9%), suggesting that aspirin can improve the oral bioavailability of dipyridamole under this condition. However, addition of fumaric acid or orange juice can also improve the oral bioavailability of dipyridamole to 68.1% or 56.4%, respectively. The results of this study may not provide any useful information since the dose of aspirin was much higher than that in Aggrenox.

Sponsor conducted a pharmacokinetic study in 12 healthy volunteers with 200 mg persantine, 25 mg aspirin and combination of 200 mg persantine and 25 mg aspirin. There were no differences in the plasma level of dipyridamole or salicylic acid when the drugs were given alone or in combination. The results were summarized in Tables 5.4.3.3:1 and 5.4.3.3:2 on pages 240 and 241 in volume 19. These tables are attached below.

TABLE 5.4.3.3: 1 Pharmacokinetic parameters and confidence intervals for DPD concentrations with treatments Persantine® ER 200 mg (P-ER) b.i.d. and Asasantin® ER 200/25 mg b.i.d. (A-ER)

Parameter	[unit]	GMean P-ER	gMean A-ER	point estimator	lower limit	Upper Limit	intraindiv. CV (%)
AUC_{ss}	[µg*h/ml]	9.59	10.4	1.08	0.97	1.21	15.2
$C_{max,ss}$	[µg/ml]	1.75	1.96	1.12	0.87	1.46	36.9

TABLE 5.4.3.3: 2 Pharmacokinetic parameters and confidence intervals for ASA and SA plasma concentrations with treatments ASA 25 mg and Asasantin® ER (200/25 mg b.i.d.)

Parameter	[unit]	GMean ASA 25 mg (reference)	gMean A-ER 200/25 mg (test)	Point estim.	lower limit	Upper Limit	CV (%)
AUC_{ss} ASA-conc.	[µg*h/ml]	0.27	0.30	1.08	0.93	1.27	21.5
AUC_{ss} SA-conc.	[µg*h/ml]	4.39	4.37	1.00	0.95	1.05	6.9

The results indicated that there is no pharmacokinetic interaction between dipyridamole and aspirin when given in the combination of dipyridamole and aspirin at a ratio of 8:1.

TOXICITY:

All toxicity studies submitted in this submission are non-GLP studies conducted prior to the promulgation of the GLP regulations in 1979 and the ratio of dipyridamole to aspirin in these studies is not the same as that (8:1) in the drug product except one acute oral toxicity study in rats (96B031).

ACUTE TOXICITY:

Methods: Acute toxicity studies were conducted in mice, rats and dogs. The dosing information was summarized in a table along with the results in the result section. These animals were observed for mortality and clinical signs of toxicity daily for up to 14 days. Body weights were recorded before and during the treatment. Necropsy was performed for gross pathological examination.

Results: The results are summarized in the following table.

Study #/Animal	Dosage (DPD/ASA, g/kg)	Mortality	Clinical Signs of Toxicity
E78-738, mice 10/sex/group 20 males at 4. g/kg	Oral (1/4.4), 1, 2, 3, 4, 5	Males: 7 at 3 g/kg, 7 at 4 g/kg, 3 at 5 g/kg Females: 1 at 1 g/kg, 2 at 4 g/kg	Prostration at 3 g/kg or higher
U77-0236, mice 5/sex/group	Oral (1/5), 1.5, 2, 2.5	1 at 1.5 g/kg, 4 at 2 g/kg, 7 at 2.5 g/kg	Shaky, tottery locomotion, convulsion at 2 and 2.5 g/kg
E78-738, mice 10/sex/group	i.p. (1/4.4), 0.25, 0.5, 0.75, 1, 1.5, 2	Males: 9 at 1 g/kg, 9 at 1.5 g/kg, 10 at 2 g/kg, Females: 3 at 1 g/kg, 10 at 1.5 g/kg, 10 at 2 g/kg	Sedation, apathy, apnea, dyspnea, prostration, tremor, coma before death
E78-738, rats 10/sex/group	Oral (1/4.4), 1, 2, 3, 4, 5	Males: 6 at 5 g/kg, Females: 2 at 4 g/kg, 5 at 5 g/kg	Prostration at all doses
96B031, rats 3/sex/group	Oral (8/1), 2/0.25, 6/0.75 ASA only: 0.75	No death	Decreased locomotor activity at all doses, prone position and piloerection at 2 combined doses
U71-0168, rats 5/sex/group	Oral (1/5): 1, 1.25, 1.6, 2, 2.5, 3.2	3 at 2 g/kg, 7 at 2.5 g/kg, 7 at 3.2 g/kg	Lethargic or restless, bleeding snouts, dragging of the hind legs before death
E78-738, rats 10/sex/group	i.p. (1/4.4), 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5	Males: 4 at 0.75 g/kg, 5 at 1 g/kg, 7 at 1.25 g/kg, 9 at 1.5 g/kg, 9 at 2 g/kg, 10 at 2.5 g/kg, Females: 3 at 1 g/kg, 6 at 1.25 g/kg, 7 at 1.5 g/kg, 9 at 2 g/kg, 10 at 2.5 g/kg	Apathy, dyspnea, prostration before death
U71-0167, dogs 1/sex/group	Oral (1/5), 0.5, 0.75, 0.875, 0.937, 1	2 at 0.937 g/kg, 2 at 1 g/kg	Sedation at all doses, lethargy followed by drowsiness at 0.5 g/kg or higher.

DPD = dipyridamole

In conclusion, treatment with combination of dipyridamole and aspirin in a ratio of 8:1 was non-lethal at doses up to 6.75 g/kg in rats and decreased locomotor activity, prone position and piloerection were observed at doses of 2.25 and 6.75 g/kg. Treatment with combination of dipyridamole and aspirin in a ratio of 1:4.5-5 produced central nervous system toxicities including sedation, prostration, and dyspnea in both mice and rats. The minimal lethal dose was 1 g/kg (oral and i.p.) in mice. The minimal lethal dose in rats was 2 g/kg (oral) and 0.75 g/kg (i.p.) in rats. The minimal lethal oral dose in dogs was 0.937 g/kg. Sedation, lethargy and drowsiness were noted in dogs.

SUBACUTE/SUBCHRONIC/CHRONIC TOXICITY:

3-Month Oral Toxicity Study with Persantine and Aspirin in Rats (U73-0185)

Testing Laboratory: [redacted]

Study Start and Completion Dates: April 24, 1972 and February 2, 1973

GLP and QAU Compliance Statement: None.

Animals: Males (150-160 g, 6 weeks old)
Females (160-170 g, 8 weeks old)
SPF rats

Methods: The oral toxicity study was conducted with combination of persantine and aspirin (1:5) in rats (20/sex/group). The oral doses of 0, 25, 100 and 400 mg/kg/day were given to rats for 3 months. The basis of dose selection was not provided. Clinical sign of toxicity was observed daily. Food consumption and body weights were recorded weekly. Hematology and clinical chemistry were not conducted. All animals were necropsied at termination. Organs were weighed and gross and histopathological examinations were performed.

Results:

1. Clinical Signs: There were no clear treatment related changes.

2. Mortality: There were no deaths.

3. Body Weight: Body weight gain was decreased by 19-23% in the high dose group.

4. Food Consumption: There were no treatment related changes.

5. Hematology: There were no treatment related changes.

6. Clinical Chemistry: There were no treatment related changes.

7. Organ Weights: There were no treatment related changes.

8. Gross Pathology: There were no treatment related changes.

9. Microscopic Pathology: There were no treatment related changes.

In summary, the combination of persantine and aspirin (1:5) was given to rats at 0, 25, 100 and 400 mg/kg/day by oral gavage for 3 months. The only treatment related changes were decreased body weight gain in the high dose group (19-23%) as compared to the control. Based on the 19-23% reduction of body weight gain, it appears that the high dose of 400 mg/kg/day was slightly higher than MTD.

24-Weeks Oral Toxicity Study with Persantine and Aspirin in Rats (U75-0196)

Testing Laboratory: Laboratories of Boehringer Ingelheim

Study Start and Completion Dates: Completed on June 27, 1978.

GLP and QAU Compliance Statement: None.

Animals: Males (195-200 g, 6 weeks old)
Females (177-181 g, 6 weeks old)
CD rats

Methods: The oral toxicity study was conducted with combination of persantine and aspirin (1:4) in rats (10/sex/group). The oral doses (esophageal tube) of persantine/aspirin of 0, 25, 100 and 400 mg/kg/day were given to rats for 24 weeks. The basis of dose selection was not provided. Clinical sign of toxicity was observed daily. Food consumption and body weights were recorded weekly. Hematology, clinical chemistry and urinalysis were conducted. All animals were necropsied at termination. Organs were weighed and gross and histopathological examinations were performed.

Results:

1. Clinical Signs: Slight salivation was noted in the high dose group.

2. Mortality: Seven animals were found dead during the study (1 control female, 1 low dose male, 4 high dose males and 1 high dose female). The control and high dose females died due to anesthesia for blood sampling. The necropsy revealed cadaveric autolysis in the low dose male and 2 high dose males and nephritis in the other high dose males.

3. Body Weight: Body weight gain was decreased by 12.5% in the high dose males as compared to the control.

4. Food Consumption: There were no treatment related changes.

5. Hematology: Bleeding time was prolonged by 129% in the high dose group during week 13.

6. Clinical Chemistry: Urea and creatinine were increased by 40 and 36% in the high dose group, respectively.

7. Urinalysis: There were no treatment related changes.

8. Organ Weights: There were no treatment related changes.

9. Gross Pathology: There were no treatment related changes.

10. Microscopic Pathology: There were no treatment related changes.

In summary, the combination of persantine and aspirin (1:4) was given to rats orally at 0, 25, 100 and 400 mg/kg/day for 24 weeks. The high dose appeared to be lethal. The treatment related changes were decreased body weight gain (12.5%), prolongation of bleeding time (129%) and increase in urea (40%) and creatinine (36%) level in the high dose group. Histopathological examination revealed no treatment related changes.

24-Week Oral Toxicity Study with Persantine and Aspirin in Dogs (U75-0196)

Testing Laboratory: Laboratories of Boehringer Ingelheim

Study Start and Completion Dates: Completed on June 27, 1978.

GLP and QAU Compliance Statement: None.

Animals: Males (6.7-15.6 kg, 7-8 months old)
Females (6.7-11.3 kg, 7-8 months old)
Beagle Dogs

Methods: The oral toxicity study was conducted with combination of persantine and aspirin (1:4) in dogs (3/sex/group). The oral doses (esophageal tube) of persantine/aspirin of 0, 25, 100, 200 and 400 mg/kg/day were given to dogs for 24 weeks. After all animals died or were sacrificed in moribund conditions at 400 mg/kg/day during 15th and 110th days of treatments, the dose of 200 mg/kg/day was introduced. The basis of dose selection was not provided. Clinical sign of toxicity was observed daily. Food consumption and body weights were recorded weekly. Hematology, clinical chemistry and urinalysis were conducted. All animals were necropsied at termination. Organs were weighed and gross and histopatholcgical examinations were performed.

Results:

1. Clinical Signs: Animals in the high dose group had "severe impairment of general health state", "reluctant to move", "a weakening of the hindquarters" and "abdominal pain" especially in the stomach region.

2. Mortality: All high dose animals died or sacrificed in moribund conditions between 15nd and 110th days of the treatment. Necropsy in these animals revealed gastritis, nephritis, discoloration of renal cortices, of spleen and of liver, and congestion of lung, pancreas, lymph nodes, and liver. One female at 200 mg/kg/day also died.

3. Body Weight: Body weight gains at 25, 100 and 200 mg/kg were comparable to the control. All animals died prematurely at 400 mg/kg.

4. Food Consumption: The high dose animals showed a fairly severe loss of appetite.

5. Hematology: The changes were minor and sporadic.

6. Clinical Chemistry: Creatinine was increased by 53-60% and 49-51% at 200 and 400 mg/kg, respectively at 1 month. At 3 months, both urea and creatinine were increased by 63-79% and 36-72% at 200 mg/kg, respectively. At the end of treatment, urea and creatinine were increased by 84-92% and 46-101% at 200 mg/kg, respectively. Creatinine was also increased by 22-31% and 10-21% at 25 and 100 mg/kg, respectively, at the end of treatment.

7. Urinalysis: Sponsor stated that there was slight albuminuria at 200 mg/kg (no data were provided).

8. Organ Weights: There were no clear treatment related changes.

9. Gross Pathology: Besides findings in the dead animals, discoloration of renal cortices, of spleen and of liver, and congestion of lung, pancreas, lymph nodes, and liver were also seen at 100 and 200 mg/kg.

10. Microscopic Pathology: Consistent with gross findings, histopathological examination revealed nephritis and congestion in the liver, spleen, lung, kidney, stomach mainly at 200 and 400 mg/kg.

In summary, the combination of persantine and aspirin (1:4) was given to dogs orally at 0, 25, 100, 200 and 400 mg/kg/day for 24 weeks. Dose of 400 mg/kg was extremely lethal and all animals died in this group. The major treatment related changes were increase in urea and creatinine by 63-92% and 36-101% at 200 mg/kg, respectively. Creatinine was also slightly increased by 22-31% and 10-21% at 25 and 100 mg/kg at the end of treatment, respectively. Histopathological examination revealed nephritis and congestions in the liver, spleen, lung, kidney, and stomach mainly at 200 and 400 mg/kg.

27-Week Oral Toxicity Study with Persantine and Aspirin in Dogs (U78-0202)

Testing Laboratory: [redacted]

Study Start and Completion Dates: July 14, 1976 and March 30, 1978

GLP and QAU Compliance Statement: None.

Animals: Males (10-13.9 kg, 9-13 months old)
Females (8.3-13.5 kg, 9-13 months old)
Beagle Dogs

Methods: The oral toxicity study was conducted with combination of persantine and aspirin (1:5) in dogs (3/sex/group). The oral doses (capsules) of persantine/aspirin of 0, 100 and 200 mg/kg/day were given to dogs for 27 weeks. Aspirin alone was also given to dogs at 80 and 160 mg/kg/day. The basis of dose selection was not provided. Clinical sign of toxicity was observed daily. Food consumption and body weights were recorded weekly. ECG, heart rate, blood pressure, respiratory rate, phonocardiogram, and sphygmogram were recorded. Hematology, clinical chemistry and urinalysis were conducted. All animals were necropsied at termination. Organs were weighed and gross and histopathological examinations were performed.

APPEARS THIS WAY
ON ORIGINAL

Results:

1. Clinical Signs: Vomiting and salivation were noted in the treatment groups including aspirin alone.

2. Mortality: There were no deaths.

3. Body Weight: The terminal body weight gains were 1.43, -0.17, and -2.83 kg (males) or 0.83, 0.13, and -1.2 kg (females) in the groups of persantine/aspirin at 0, 100, 200 mg/kg/day, respectively. The terminal body weight gains were 0.73 and 0.67 kg (males) or 1.03 and -0.03 kg (females) in groups of aspirin alone at 80 and 160 mg/kg/day, respectively.

4. Food Consumption: There were no clear treatment related changes.

5. Ophthalmology: There were no treatment related changes.

6. ECG, Heart Rate and Blood Pressure: Heart rate was increased by 30% and 39-57% in the groups of persantine/aspirin of 100 and 200 mg/kg/day associated with decreased blood pressure (11-15%). These effects were observed at 2 hours after dosing and gradually disappeared. The decreased blood pressure could be due to peripheral vasodilation of persantine, which in turn increased the heart rate. There were no clearly treatment related changes in ECG.

7. Phonocardiograms, Sphygmogram and Respiratory Rate: There were no clear treatment related changes.

8. Hematology: The mean values of the hematological parameters were within normal range during almost all times of the treatment period. Some sporadic changes were not clearly treatment related.

9. Clinical Chemistry: Some sporadic changes were not clearly treatment related.

10. Urinalysis: There were no treatment related changes.

11. Organ Weights: Heart and kidney weights were increased by 8.7% and 24% in the group of persantine/aspirin at 200 mg/kg/day, respectively. The kidney weight was also increased by 15% and 23% in the groups of persantine/aspirin at 100 mg/kg/day and aspirin alone at 160 mg/kg/day, respectively. The spleen weight was decreased by 28% and 35% in groups of persantine/aspirin at 100 and 200 mg/kg/day, respectively, and increased in one female in the group of aspirin alone at 160 mg/kg/day (112 g vs mean of 33.5 g in control group).

12. Gross Pathology: Erosive changes in gastric mucosa were seen in animals in all treatment groups. Greyish striations of the renal cortex were noted in 2 animals treated with combination of persantines and aspirin at 100 mg/kg/day, 1 animal treated with combination of persantine and aspirin at 200 mg/kg/day and 2 animals treated with aspirin alone at 160 mg/kg/day. Three animals treated with combination of persantine and aspirin at 200 mg/kg/day had ventricular hypertrophy and one of these animals also showed disseminated greyish-yellow and greyish-white foci in the subendocardial region of papillary muscle.

13. Microscopic Pathology: Consistent with gross findings, histopathological examination revealed erosions of the gastric mucosa, fat content in the epithelium of the proximal renal tubules, tubular atrophy in all treatment groups. Scar tissues in the ventricular papillary muscle were noted in the groups of combination of persantine and aspirin at 100 and 200 mg/kg/day and aspirin alone at 160 mg/kg/day. In the group of combination of persantine and aspirin at 200 mg/kg/day, following changes were also noted: moderate, diffuse, non-degenerative fatty change in the myocardium (1 animal), focal, intervillius hemorrhages in the jejunal mucosa (1 animal), extensive subacute to chronic penetrating ulcer in the ileum (1 animal), slight to moderate non-degenerative fatty change in the parenchyma of the liver (2 animals), subacute to chronic granulomatous arteritis and periarteritis in the testes and epididymes (3 animals).

In summary, dogs were treated orally with the combination of persantine and aspirin (1:5) at 0, 100 and 200 mg/kg/day and aspirin alone at 80 and 160 mg/kg/day for 27 weeks. The treatment with combination of persantine and aspirin produced cardiac toxicity as evidenced by increased heart rate and histopathological changes including ventricular hypertrophy and scar tissues in the ventricular papillary muscle. Pathological changes were also seen in the liver, kidney, stomach, small intestine, testes and epididymes. Therefore, the target organs of toxicity were the heart, kidney, stomach, small intestine, liver, testes and epididymes.

CARCINOGENICITY:

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC)
RODENT CARCIOGENICITY FACTSHEET

NDA: 20,884
CAS #:
DIVISION(s): HFD-180
DRUG NAME(s): AGGRENOX
SPONSOR: Boehringer Ingelheim pharmaceuticals, Inc.
LABORATORY: [redacted]

P/T REVIEWER(s): Ke Zhang, Ph.D.
P/T REVIEW DATE: April 23, 1999
CARCINOGENICITY STUDY REPORT DATE: February 20, 1979
THERAPEUTIC CATEGORY:
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Antiplatelet agents
PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No
MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): Mutagenicity testing with combination of dipyridamole and aspirin in a 1:5 ratio revealed no mutagenic potential in Ames test, in vivo chromosome aberration tests in mice and hamsters, oral micronucleus tests in mice and hamsters and dominant lethal test in mice.

MOUSE CARCINOGENICITY STUDY (multiple studies? Std1, Std2 etc):

MOUSE STUDY DURATION (weeks): 105 (males and females)
STUDY STARTING DATE: November 4, 1975
STUDY ENDING DATE: December 9, 1977
MOUSE STRAIN: NMRI mice
ROUTE: Oral (in feed)
DOSING COMMENTS:
No. Mice in Control (C): 100m,100f
Low Dose (LD): 50m,50f **Middle Dose (MD):** 50m,50f
High Dose (HD): 50m,50f

MOUSE DOSE LEVELS (mg/kg/day): Ratio of dipyridamole and asprin = 1:5

Low Dose: 50 **Mid Dose:** 150 **High Dose:** 450

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): Not provided

MOUSE CARCINOGENICITY (negative, positive, MF, M, F): Negative (MF)

MOUSE TUMOR FINDINGS: None

MOUSE STUDY COMMENTS: In this oral carcinogenicity study in mice, combination of dipyridamole and aspirin (1:5) was given to mice in feed at 0, 50, 150 and 450 mg/kg/day for 105 weeks. No toxicity was identified at doses up to the high dose, suggesting that the animals were not exposed to sufficient high doses. The mortality and tumor rate were comparable in all groups. There was no treatment related tumorigenicity.

Oral (in feed) 105-Week Toxicity Study with Combination of Persantine and Aspirin in Mice
(U79-0257)

Testing Laboratory: [redacted]

Study Start and Completion Dates: November 4, 1975 and February 20, 1979

GLP and QAU Compliance Statement: None.

Animals: Males (24-25 g, 17 days old)
Females (21-22 g, 18 days old)
NMRI mice

Methods: The original purpose of this study was to investigate the oral (in feed) tolerance of a combination of persantine and aspirin in mice. Sponsor also stated that "particular attention was paid to possible carcinogenic properties". In this study, mice (100/sex/control, 50/sex/group) were treated with combination of persantine and aspirin (1:5) at 0, 50, 150 and 450 mg/kg/day in feed for 105 weeks. Basis of dose selection was not provided. Clinical sign of toxicity was observed daily. Food and water consummations and body weights were recorded. Ophthalmoscopic examination and hearing test were conducted at termination. The surviving animals were necropsied at termination. Gross and histopathological examinations were performed in all animals and organs were weighed. The organs or tissues examined histopathologically were listed on page 172 in volume 30 and this list is attached below.

heart	brain	bladder
lung	prostate/uterus	bone marrow
liver	stomach	trachea
spleen	duodenum	aorta
kidney	jejunum	oesophagus
adrenal	ileum	pancreas
thymus	colon	mesenteric lymph nodes
pituitary	rectum	peripheral nerve
gonads	salivary gland	skeletal muscle
thyroid	eye and optic nerve	bones
		tumours

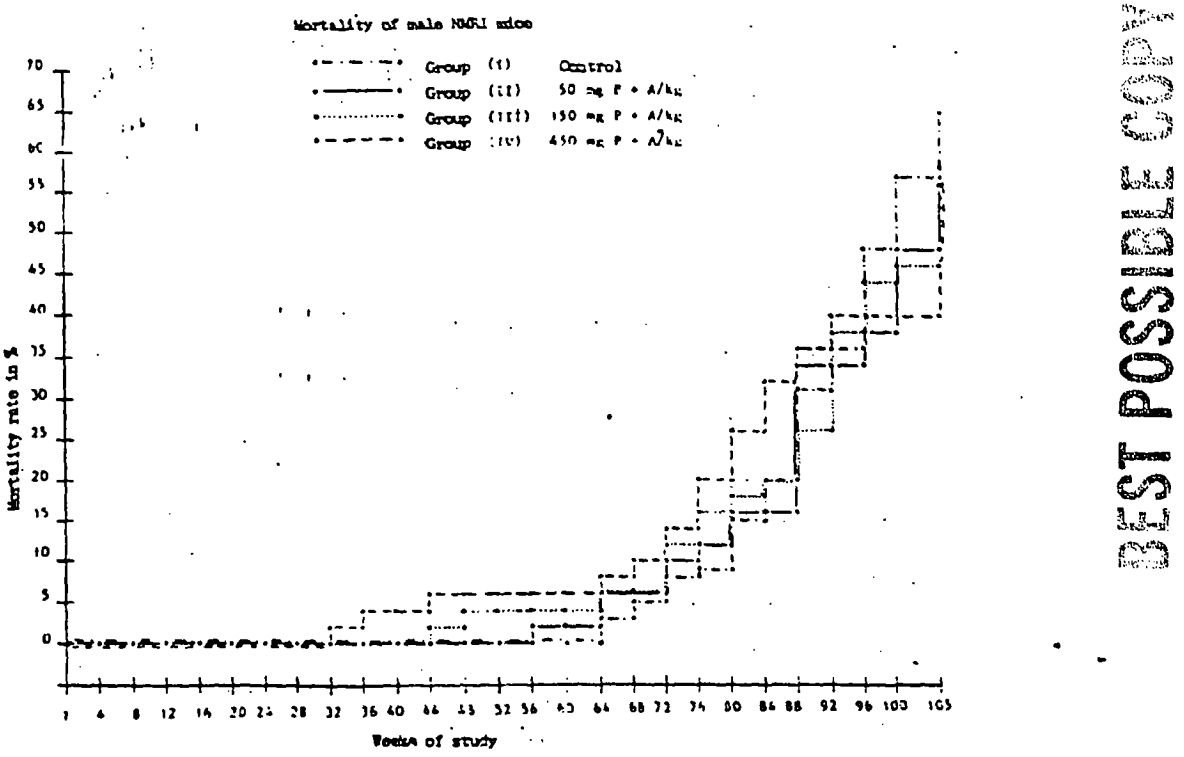
The tumor and mortality rates were compared statistically using the analysis of variance of PETO ("Guidelines on the analysis of tumor rates and death rates in experimental animals" published in Brit. J. Cancer 1974, 29:101-105). All other parameters were analyzed statistically using Student's t-test.

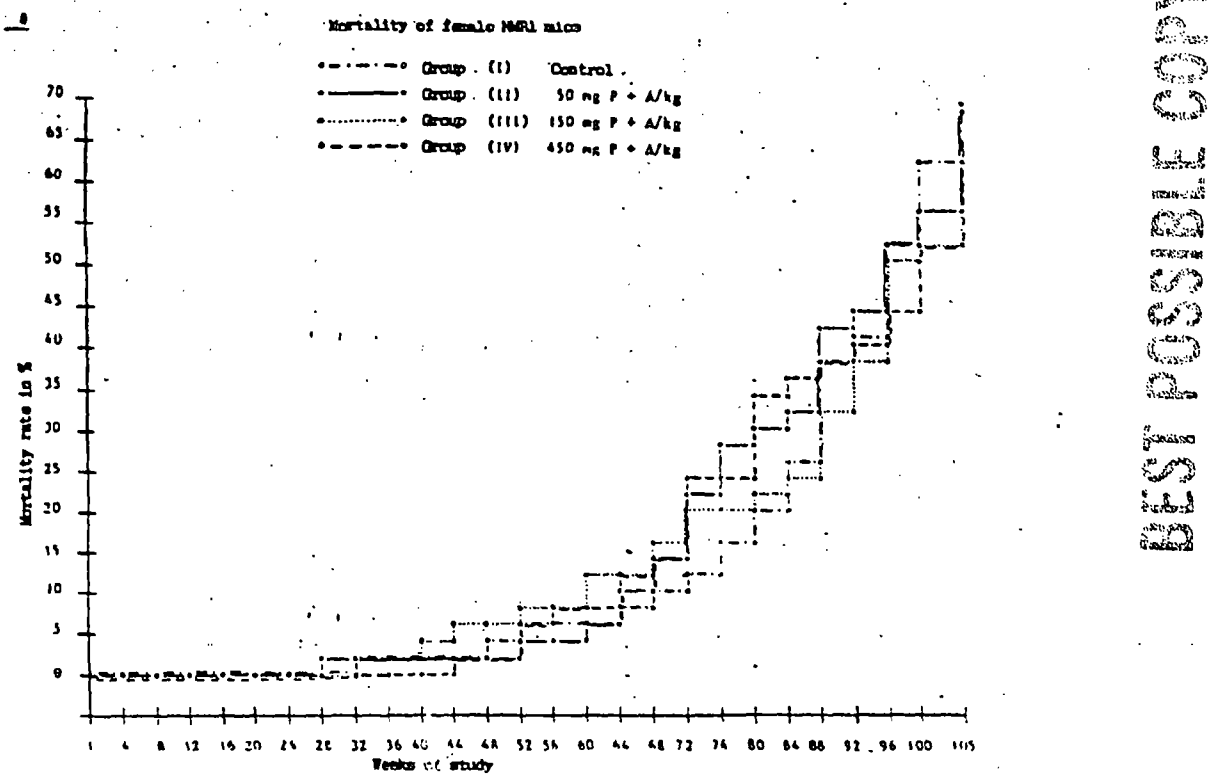
Results: No treatment related clinical signs of toxicity were observed. Mortality was comparable in all groups and this was summarized on page 174 in volume 130 and this is attached below.

P + A dose in mg/kg B.W. in the food	Mortality rate in %		
	m	f	m/f*
control (untreated)	65	69	67
50	56	68	62
150	58	56	57
450	56	64	60

*combined mean value of male (m) and female (f) mice.

Mortality data were also depicted in Figures 7 and 8 on page 365 and 366 in volume 30. These figures are attached below.





The body weights at start and week 105 are summarized in the following table.

Body Weight (g)

	Control	Low dose	Mid dose	High dose
Initial				
Male	23.8 ± 1.9	24.7 ± 1.5	24.7 ± 1.7	24.0 ± 1.9
Female	21.8 ± 1.7	22.1 ± 1.7	22.3 ± 1.6	21.2 ± 1.4
Week 105				
Male	42.5 ± 4.2	42.7 ± 4.1	42.6 ± 3.5	41.8 ± 4.0
Female	36.1 ± 3.7	34.9 ± 4.1	35.5 ± 4.8	33.8 ± 4.5

The terminal body weights in the low, mid and high dose groups was 100.5%, 100.2% and 98.4% (males) or 96.7%, 98.3% and 93.6% (females) of the control, respectively. The growth curves were depicted in Figures 5 and 6 on pages 363 and 364 in volume 30 and these figures are attached below.

TABLE 5.

Body weight of male NMRI mice

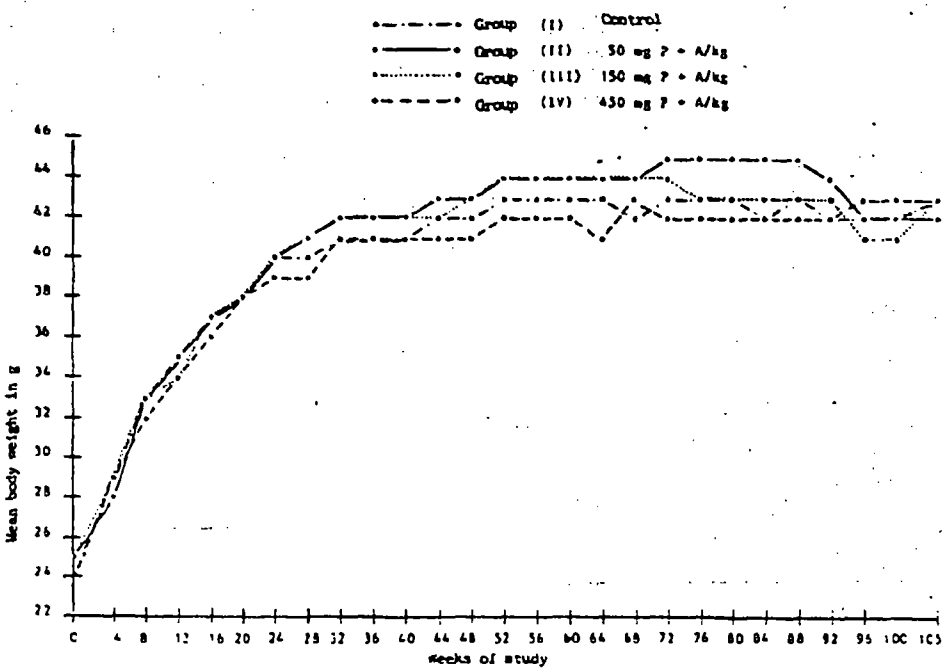
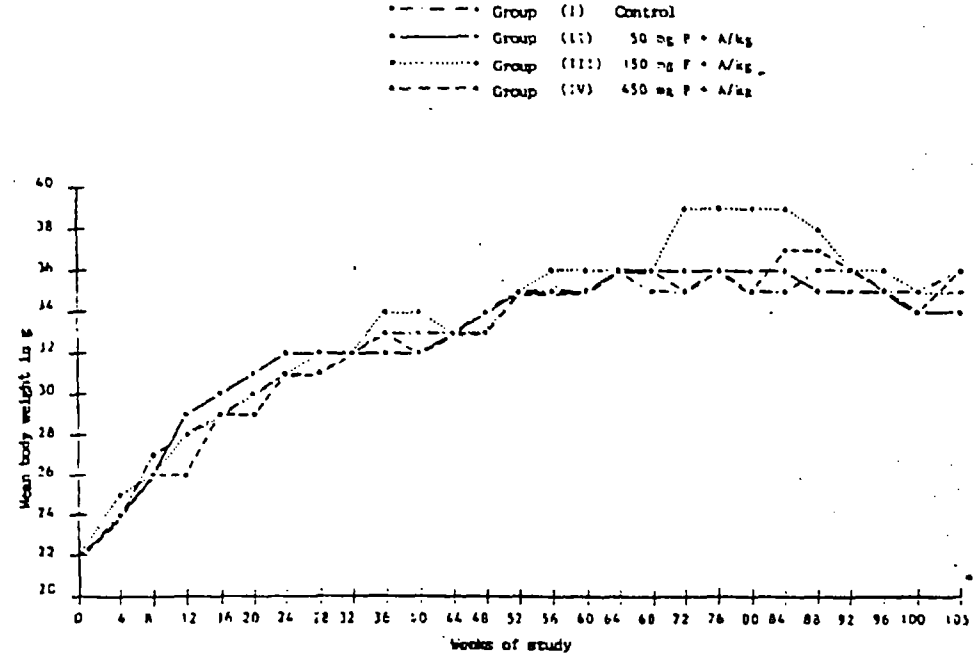


TABLE 6.

Body weight of female NMRI mice



Water and food consumptions were comparable in all groups. The actual drug intake and the daily food consumption was summarized on page 175 in volume 30 and this is attached below.

Group	males
II	49.4 mg P + A/kg B.W. ± 3.7 mg/kg
III	148.4 mg P + A/kg B.W. ± 10.0 mg/kg
IV	444.7 mg P + A/kg B.W. ± 32.2 mg/kg

Group	females
II	49.7 mg P + A/kg B.W. ± 3.2 mg/kg
III	149.2 mg P + A/kg B.W. ± 9.9 mg/kg
IV	448.2 mg P + A/kg B.W. ± 31.4 mg/kg

Ophthalmoscopic examination and hearing test revealed no abnormality. No treatment related histopathological changes were found. The tumor rate (%) is summarized in the following table.

Tumor Rate (%)

	Control	Low Dose	Mid Dose	High Dose
Male	30	42	32	32
Female	47	48	56	36
Mean	38.5	45	44	34

The individual tumor incidence was summarized in Table 10 on page 195 in volume 30 and this table is attached in Appendix I.

In conclusion, treatment with combination of persantine and aspirin in feed (1:5) for 105 weeks did not produce any toxicity at doses up to the high dose of 450 mg/kg/day. The basis of dose selection was not provided. The mortality was comparable in all groups. There was no treatment related tumorigenicity.

FDA CDER CARCINOGENICITY ASSESSMENT COMMITTEE (CAC/CAC-EC) RODENT CARCIOGENICITY FACTSHEET

NDA: 20,884
CAS #:
DIVISION(s): HFD-180
DRUG NAME(s): AGGRENOX
SPONSOR: Boehringer Ingelheim Pharmaceuticals, Inc.
LABORATORY: [redacted]

P/T REVIEWER(s): Ke Zhang, Ph.D.
P/T REVIEW DATE: April 23, 1999
CARCINOGENICITY STUDY REPORT DATE: February 6, 1979
THERAPEUTIC CATEGORY:
PHARMACOLOGICAL/CHEMICAL CLASSIFICATION: Antiplatelet agents
PRIOR FDA DOSE CONCURRENCE (Div./CAC)? (Y/N; Date): No
MUTAGENIC/GENOTOXIC (Y/N/equivocal/na; assay): Mutagenicity testing with combination of dipyridamole and aspirin in a 1:5 ratio revealed no mutagenic potential in Ames test, in vivo chromosome aberration tests in mice and hamsters, oral micronucleus tests in mice and hamsters and dominant lethal test in mice.

RAT CARCINOGENICITY STUDY (multiple studies? Std1, Std2 etc):

RAT STUDY DURATION (weeks): 125 (males and females)
STUDY STARTING DATE: August 8, 1975
STUDY ENDING DATE: January 14, 1978
RAT STRAIN: Chbb:THOM rats
ROUTE: Oral (in feed)
DOSING COMMENTS:

No. Rats in Controll (C): 100m,100f
Low Dose (LD): 50m,50f **Middle Dose (MD):** 50m,50f
High Dose (HD): 50m,50f

RAT DOSE LEVELS (mg/kg/day): Ratio of dipyridamole and asprin = 1:5

Low Dose: 50 **Mid Dose:** 150 **High Dose:** 450

Basis for Doses Selected (MTD; AUC ratio; saturation; maximum feasible): Not provided

RAT CARCINOGENICITY (negative, positive, MF, M, F): Negative (MF)

RAT TUMOR FINDINGS: None

RAT STUDY COMMENTS: In this oral carcinogenicity study in rats, combination of dipyridamole and aspirin (1:5) was given to rats in feed at 0, 50, 150 and 450 mg/kg/day for 125 weeks. No toxicity was identified at doses up to the high dose except lower terminal body weight in the high dose females (85% of the control). It appears that the males were not exposed to sufficient high doses. There was no treatment related tumorigenicity.

Oral (in feed) 125-Week Toxicity Study with Combination of Persantine and Aspirin in Rats
(U79-0258)

Testing Laboratory:

Study Start and Completion Dates: August 8, 1975 and February 6, 1979

GLP and QAU Compliance Statement: None.

Animals: Males (105-127 g, 40-50 days old)
Females (103-126 g, 40-46 days old)
Chbb:THOM rats

Methods: The original purpose of this study was to investigate the oral (in feed) tolerance of a combination of persantine and aspirin in rats with particular attention to possible carcinogenic properties. In this study, rats (100/sex/control, 50/sex/group) were treated with combination of persantine and aspirin (1:5) at 0, 50, 150 and 450 mg/kg/day in feed for 125 weeks. Basis of dose selection was not provided. Clinical sign of toxicity was observed daily. Food and water consumptions and body weights were recorded. Ophthalmoscopic examination and hearing test were conducted at termination. The surviving animals were necropsied at termination. Gross and histopathological examinations were performed in all animals and organs were weighed. The organs or tissues examined histopathologically were listed on page 19 in volume 31 and this list is attached below.

heart	brain	bladder
lung	prostate/uterus	bone marrow
liver	stomach	trachea
spleen	duodenum	aorta
kidney	jejunum	oesophagus
adrenal	ileum	pancreas
thymus	colon	mesenteric lymph nodes
pituitary	rectum	peripheral nerve
gonads	salivary gland	skeletal muscle
thyroid	eye and optic nerve	bones
		tumours

The tumor and mortality rates were compared statistically using the analysis of variance of PETO ("Guidelines on the analysis of tumor rates and death rates in experimental animals" published in Brit. J. Cancer 1974, 29:101-105). All other parameters were analyzed statistically using Student's t-test.

Results: No treatment related clinical signs of toxicity were observed. Mortality was comparable in all groups and this was summarized in a table on page 21 in volume 131. This table is attached below.

P + A dosage	Mortality rate in %		
mg/kg body weight in the food	m	f	m/f*
control (no dose)	54	53	53.5
50	68	52	60.0
150	70	48	59.0
450	52	58	55.0

*combined mean value of male (m) and female (f) animals.

Mortality data were also depicted in Figures 7 and 8 on page 270 and 271 in volume 31. These figures are attached below.

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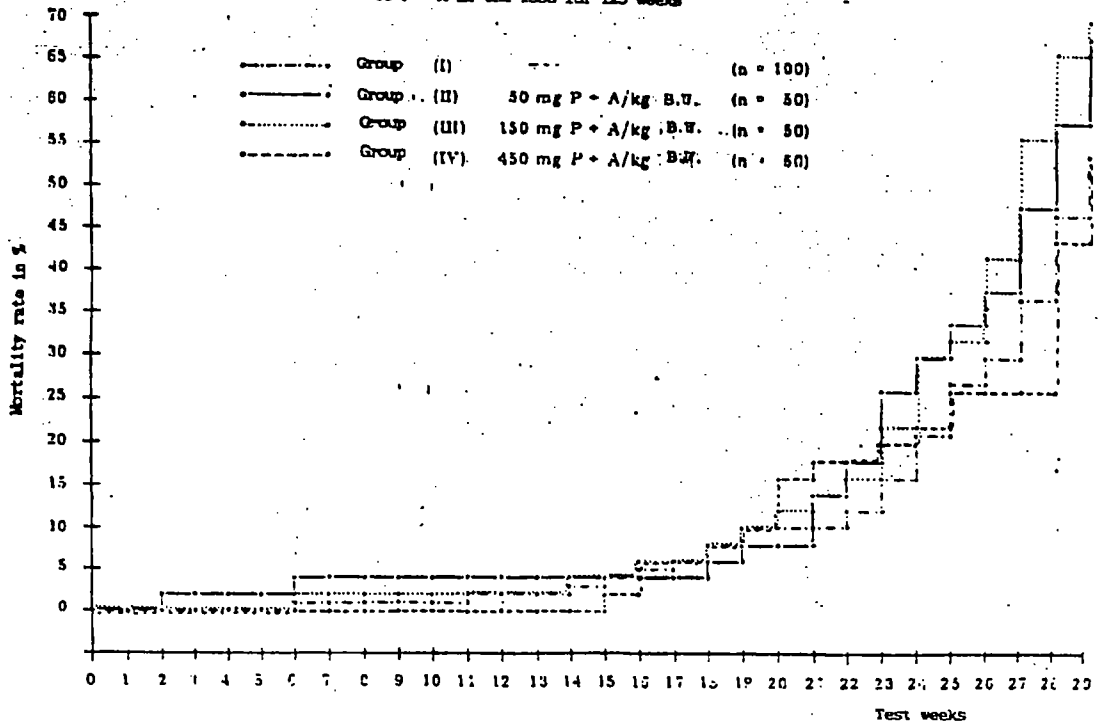
ON ORIGINAL

FIGURE 7

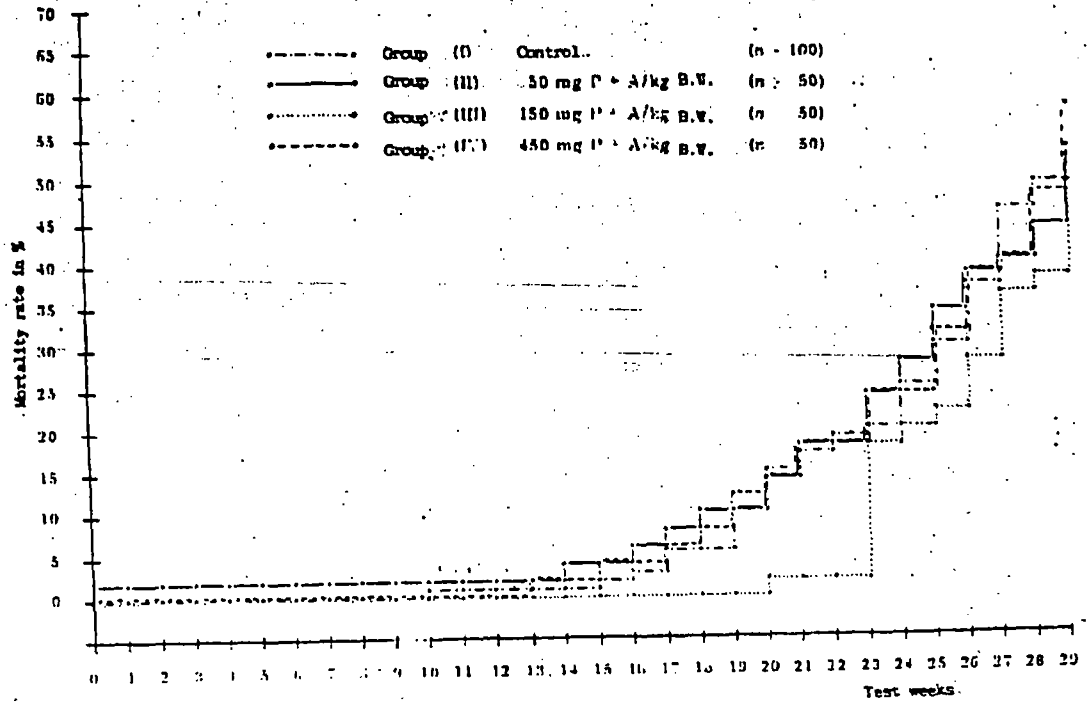
Mortality of male CrlbR:THOM rats with administration of P + A in the food for 125 weeks



APPEARS THIS WAY
ON ORIGINAL

FIGURE 8

Mortality of female Chbb:THOM rats with administration of P + A in the food for 125 weeks



The body weights at start and week 125 are summarized in the following table.

Body Weight (g)

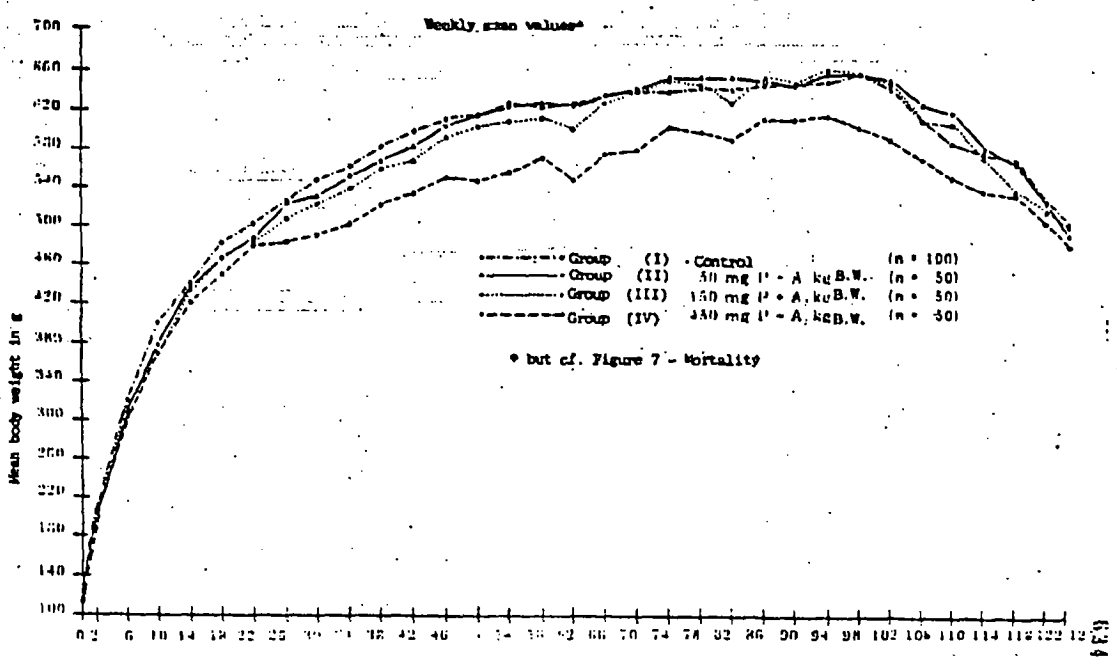
	Control	Low Dose	Mid Dose	High Dose
Initial				
Male	114.5±5.4	116.1±5.0	113.9±5.2	115.5±5.1
Female	114.3±5.0	113.3±5.9	115.5±4.9	114.8±5.3
week 125				
Male	504.5±71.7	492.3±71.8	501.8±60.5	481.1±54.5
Female	359.1±56.0	359.1±60.3	341.3±63.6	304.9±40.7

The terminal body weights in the low, mid and high dose groups were 97.6%, 99.5% and 95.4% (males) or 100%, 95% and 84.9% (females) of the control, respectively. The growth curves were depicted in Figures 5 and 6 on pages 268 and 269 in volume 31 and these figures are attached below.

FIGURE 5

Body weight of male Cbb:TRON rats with administration of P + A in the food for 125 weeks

Weekly mean values*



* but cf. Figure 7 - Mortality

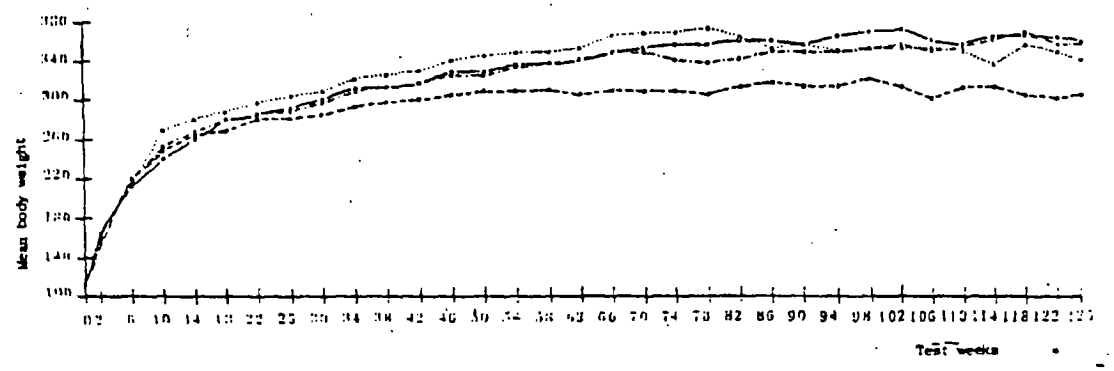
FIGURE 6

Body weight of female Cbb:TRON rats with administration of P + A in the food for 125 weeks

Weekly mean values

Group (I) Control (n = 100)
Group (II) 50 mg P + A kg B.W. (n = 50)
Group (III) 150 mg P + A kg B.W. (n = 50)
Group (IV) 450 mg P + A kg B.W. (n = 50)

* but cf. Figure 8 - Mortality -



Water and food consumptions were not affected by the treatment. The actual drug intake calculated using the daily food consumption is summarized in the following table.

Mean drug intake (mg)

	Low Dose	Mid Dose	High Dose
Male	49.5±5.1	147.7±12.3	443.8±38.4
Female	49.5±4.2	148.7±14.0	446.9±39.6

Ophthalmoscopic examination and hearing test revealed no abnormality. No treatment related histopathological changes were found. The tumor rate (%) is summarized in the following table.

Tumor Rate (%)

	Control	Low Dose	Mid Dose	High Dose
Male	79	74	66	50
Female	66	66	68	76
Mean (male & female)	72.5	70	67	63

The individual tumor incidence was summarized in Table 11 on page 262 in volume 31 and this table is attached in Appendix I.

In conclusion, treatment with combination of persantine and aspirin in feed (1:5) for 125 weeks did not produce any toxicity at doses up to the high dose of 450 mg/kg/day in rats except lower terminal body weight in the high dose females (85% of the control). Basis of dose selection was not provided. There was no treatment related tumorigenicity.

REPRODUCTIVE TOXICITY:

An Oral Teratogenic Study of Combination of Persantine and Aspirin in Rats
(U78-0203)

Testing Laboratory: [redacted]

Dates Started and Completed: September 20, 1977 and October 21, 1977

GLP and QAU Compliance Statement: None.

Animals: Female (~227 g, 3 months old)
Sprague Dawley rats

Methods: To study the potential teratogenic effects of combination of persantine and aspirin in rats, rats was given orally combination of persantine and aspirin (1:4.4) at 0, 50.62, 202.5, and 405 mg/kg/day or aspirin alone at 330 mg/kg/day to pregnant female rats from gestation days 6 to 15. All animals were observed daily for clinical signs of toxicity and mortality. Body weights and food consumption were recorded. Females were sacrificed on day 21 of pregnancy. Number of corpora lutea and implantation sites, live and dead fetuses, fetal and placental weights and fetal sex were recorded. All live fetuses were examined externally. Fetuses were also examined for visceral and skeletal alterations.

Results: There were no deaths in this study. No treatment related clinical signs of toxicity were observed. The body weight gain was decreased in the treatment groups (14%, 29%, 43% and 47% in low, mid and high dose groups and aspirin alone group, respectively) as compared to the control. The fetal information was summarized in Table VI on page 340 in volume 28 and this table is attached below.

TABLE V:

Summary of the observations made at autopsy of the gravid females

(Group means)

Groups	Number of impregnated rats	Number of implantations	No. of resorptions: Early	Late	total	Abortions	Total no. of fetuses	Sex: M	F	Mean no. of fetuses per litter	Mean weight of one litter (g)	Mean weight of one fetus (g)	% resorptions in relation to % of implantations
Control	16	193	8	2	10	0	183	102	81	11,4	63,208	5,264	5,18 %
A	18	222	8	1	9	1	213	108	105	12,5	63,022	5,029	4,05 %
B	17	225	5	0	5	0	220	103	117	12,9	59,847	4,624	2,22 %
C	18	204	70	133	203	17	1	0	1	1	1,709	1,709	99,50 %
D	21	259	85	79	164	8	95	49	46	7,3	23,554	3,228	63,32 %

M : males
F : females

Abortion occurred in the low (1/18) and high (17/18) dose groups and aspirin treatment group (8/21). No abortion was noted in the control and mid dose groups. Fetus weight was markedly decreased in the mid (12%) and high (68%) dose groups and aspirin group (39%) as compared to the control.

Fetal examination revealed small size fetuses each in the low, mid and high dose groups and 14 in the aspirin group. In the aspirin group, following external anomalies were noted: spina bifida (20 fetuses), exencephaly (26), microphthalmia (7), coelosomia (3), and short hind leg (1). There was a total of 95 fetuses in the aspirin group. The results of skeletal examination were summarized in the following table.

Groups	Skeletal Findings
Low dose	13 pairs of ribs (all fetuses), absence of 5th sternebra (3 fetuses), 5th sternebra bipartite (1 fetus)
Mid dose	13 pairs of ribs (all fetuses), absence of 5th and 6th sternebra (3 fetuses), all sternebrae bipartite (1 fetus)
High dose	Only a small size fetus in this group
Aspirin	Absence of all the sternebra (17 fetuses), absence of 5th sternebra (5 fetuses), absence of 4th and 5th sternebra (2 fetuses), all sternebra bipartite (13 fetus), fused ribs (9 fetuses), and 1st, 2nd and 3^{rs} sternebra bipartite (1 fetus)

Additional malformations found in the aspirin group included large space between the frontal and parietal bones (1 fetus), absence of one side of skull, frontal bone and parietal bone (1 fetus), absence of the frontal, pariental, interparietal and the occipital bones (or spina bifida in 20 fetuses).

In summary, pregnant rats were treated orally with combination of persantine and aspirin (1:4.4) at 0, 50.62, 202.5, and 405 mg/kg/day or aspirin alone at 330 mg/kg/day to pregnant female rats. Maternal toxicity was noted in the treatment groups as evidenced by decreased body weight (14%, 29%, 43% and 47% in the low, mid and high dose groups and aspirin alone group, respectively). Treatment with combination of persantine and aspirin induced abortion (1/18 in low dose group and 17/18 in high dose group) and decreased fetus weight in the mid (12%) and high (68%) dose groups. No teratogenic evidence was found in the animals treated with the combination of persantine and aspirin. Treatment with aspirin produced a number of major malformations and thus was teratogenic.

An Oral Teratogenic Study of Combination of Persantine and Aspirin in Rabbits
(U78-0204)

Testing Laboratory: [redacted]

Dates Started and Completed: September 28, 1977 and October 12, 1977

GLP and QAU Compliance Statement: None.

Animals: Female (mean weight = 3 kg, age not specified) New Zealand rabbits

Methods: To study the potential teratogenic effects of combination of persantine and aspirin in rabbits, rabbits were given combination of persantine and aspirin (1:4.4) by esophageal tube at 0, 27, 81, and 135 mg/kg/day or aspirin alone at 110 mg/kg/day to pregnant female rats from gestation days 6 to 18. All animals were observed daily for clinical signs of toxicity and mortality. Body weights and food consumption were recorded. Females were sacrificed on day 28 of pregnancy. Number of corpora lutea and implantation sites, live and dead fetuses, fetal and placental weights and fetal sex were recorded. All live fetuses were examined externally. Fetuses were also examined for visceral and skeletal alterations.

Results: Two mid dose animals died and perforating ulcer and peritonitis were noted in these animals. One high dose animal was sacrificed on day 21 and histopathological examination revealed acute epithelial nephritis in this animal. The body weight gain in the high dose animals was only ~1/3 of the control animals. Some mid dose and all high dose animals ceased eating. The fetal information was summarized in Table IV on page 34 in volume 29 and this table is attached below.

Table IV

SUMMARY OF THE OBSERVATIONS MADE AT AUTOPSY OF THE GRAVID FEMALES

(Group means)

Groups	Number of gravid rabbits (1)	Number of implantations	Number of resorptions: Early	Late	Total	Total number of fetuses	Sex: M	F	Mean number of fetuses per litter	Mean weight of one litter (g)	Mean weight of one fetus (g)	Mean weight of one placenta (g)	Fetal loss
Control	12	125(2)	3	7	10	115	63	52	9,5	331,63	34,60	5,48	8 %
A	13	113	7	4	11	102	60	42	7,8*	278,89*	35,64	6,15	9,7 %
B	10	99	0	2	2	97	47	50	9,7	359,11	37,02***	5,19	2,02%
C	12	103	1	14	15	88	45	43	7,3*	205,59***	28,03***	4,89	15,5 %
D	11	113	2	6	8	105	60	45	9,5	328,37	34,45	5,24	13,7 %

(1) Up to day 28
(2) Including the female which aborted

* p < 0.05
** p < 0.01
*** p < 0.001

Fetus weight was decreased by ~19% in the high dose group.

Small size fetuses were seen in all groups including control and the incidence of small size fetuses was not treatment related. One high dose animal had coelosomia and one case of spina bifida was reported in a high dose animal. In the aspirin group, following external anomalies were noted: congested fetuses (10 fetuses), agenesis of skull and upper jaw (1 fetus), generalized edema with malformation of the head (1 fetus), and daiphanous skin (3 fetuses). The results of skeletal examination were summarized in the following table.

Groups	Skeletal Findings
Control	13 pairs of ribs (52 fetuses), absence of 5th sternebra (15 fetuses), absence of 6th sternebra (9 fetuses), absence of 5th and 6th sternebra (5 fetuses),
Low dose	13 pairs of ribs (34 fetuses), absence of 5th sternebra (3 fetuses), absence of 6th sternebra (6 fetuses), absence of 5th and 6th sternebra (7 fetuses), 5th sternebrae bipartite (1 fetus)
Mid dose	13 pairs of ribs (43 fetuses), absence of 5th sternebra (6 fetuses), absence of 6th sternebra (8 fetuses), absence of 5th and 6th sternebra (2 fetuses), sternebra bipartite (5 fetus)
High dose	13 pairs of ribs (40 fetuses), absence of 5th sternebra (5 fetuses), absence of 6th sternebra (14 fetuses), absence of 5th and 6th sternebra (9 fetuses), absence of 2nd, 3rd and 5th sternebra (4 fetuses), sternebra bipartite (7 fetus)
Aspirin	Absence of all the sternebra (13 fetuses), absence of 5th sternebra (6 fetuses), absence of 6th sternebra (9 fetuses), absence of 5th and 6th sternebra (4 fetuses), sternebra bipartite (1 fetus)

Incomplete frontal bone (4 high dose fetuses), incomplete ossification of frontal and parietal bones (1 fetus in aspirin group) and absence of entire upper part of the skull (1 fetus in aspirin group) were also found.

In summary, pregnant rabbits were treated orally with combination of persantine and aspirin (1:4.4) at 0, 27, 81, and 135 mg/kg/day or aspirin alone at 110 mg/kg/day to pregnant female rabbits from gestation days 6 to 18. Reduction of body weight gain was noted in the high dose group. One high dose animal had coelosomia and one case of spina bifida was reported in a high dose animal. In the aspirin group, following external anomalies were noted: congested fetuses (10 fetuses), agenesis of skull and upper jaw (1 fetus), generalized edema with malformation of the head (1 fetus), and diaphanous skin (3 fetuses). No clear teratogenic evidence was found in the animals treated with the combination of persantine and aspirin. However, aspirin induced a number of major malformations and thus was teratologic in this study.

Malformations associated with aspirin were also reported previously in animals. These included cleft lip, execephaly, microcephaly, and spina bifida in mice (Trasler, 1965, Lancet, 1:606), hydrocephalus, and cleft palate in rats (Kimmel, et al., 1971, Teratology, 4:15, McColl et al., 1965, Toxicol. Appl. Pharmacol. 7:409), heart and rib defects in rabbits (McColl et al., 1967, Toxicol. Appl. Pharmacol., 10:244), cleft palate, micrognathia, anasarca, cardiovascular malformations and tail anomalies in dogs (Robertson et al., 1979, Teratology, 20:31), and heart defects in rhesus monkeys (Wilson, 1971, Fed. Proc., 30:104).

MUTAGENICITY TOXICITY STUDIES:

Mutagenicity Studies of the Combination of Persantine and Acetylsalicylic Acid (ASA) on Microorganisms (U78-0206)

Testing Laboratory: [redacted]

Dates Started and Completed: May 18, 1976 and January 31, 1978

GLP and QAU Compliance Statement: None.

Methods: To examine the potential mutagenic effects of combination of persantine and ASA, three mutation assays were conducted (hosted mediated assay, blood mediated assay and plate incorporation assay). In the host-mediated assay, combination of persantine/ASA (1:5) was given to mice by gastric tube at 0, 100 and 1000 mg/kg followed by i.p. injection of 2 ml bacterial suspension (S. Typhimurium TA 1530 and S. Typhimurium G46). Three hours later, these animals were sacrificed, abdominal cavity was opened, and washed with physiological saline. To recover the bacteria in the abdominal cavity, the fluid in the cavity was collected and placed in medium. These places were then incubated at 37° C for 24 and 72 hours and the colonies were counted. The positive control, dimethylnitrosamine (100 mg/kg), was also given to mice. In the blood-mediated assay, 0.25 ml bacterial suspension (E. coli 343/113) was given intravenously to mice and then these animals were treated with combination of persantine/ASA by gastric tube at 0, 100 and 1000 mg/kg. Two hours later, these animals were sacrificed and liver was removed and homogenized. The supernatant (in physiological saline) of the homogenate was placed in medium and incubated at 37° C for 24, 48, and 65 hours. The colonies were then counted. The direct plate incorporation mutation assay (Ames test) was conducted in four strains of S. typhimurium (TA100, TA1535, TA1537 and TA 1538) in the presence and absence of metabolic activation, S-9 mix from rat liver. The following concentrations of the combination of persantine and ASA were tested: 100, 500, and 1000 µg/plate. Vesicle and positive controls (β-naphthylamine, benzo(a) pyrene, benzidine) were also tested.

Results: The results indicated that treatment with combination of persantine and ASA did not significantly increase the colonies in all three assays. However, the positive controls significantly increased the colonies compared to the solvent controls.

In conclusion, the results suggest that persantine and ASA in combination were not mutagenic in these test systems.

Cytogenetic Evaluation of Combination of Persantine and Acetylsalicylic Acid (ASA) in Mice and Hamster
(U78-0201)

Testing Laboratory: [redacted]

Dates Started and Completed: August 11, 1976 and January 31, 1978

GLP and QAU Compliance Statement: None.

Methods: To examine the potential induction of chromosomal aberrations by combination of persantine and ASA, the *in vivo* chromosomal aberration test was conducted in mice and Chinese striped hamsters. Combination of persantine and ASA (1:5) was given to mice or hamsters by gastric tube twice with a 24 hour interval at 0, 10, 100, and 1000 mg/kg. Hamsters were sacrificed 6 or 24 hours after the last dose and bone marrow and germ cells were prepared. Mice were sacrificed 24 or 48 hours after the last dose and germ cells were prepared (no details were provided). These preparations were evaluated for chromosomal aberrations. Positive control (cyclophosphamide, 60 mg/kg) was also tested. Colcemid (4 or 5 mg/kg, i.p.) was given mice and hamsters 2.5 or 5.5 hours before sacrifice, respectively.

Results: Treatment with combination of persantine and ASA did not significantly increase the frequency of the chromosomal aberration in both preparations from mouse (germ cells) and hamster (bone marrow and germ cells). The positive controls significantly increased it.

In conclusion, the results suggest that treatment with combination of persantine and ASA was not cytogenetic in these test systems.

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Oral Micronucleus Test with Combination of Persantine and Acetylsalicylic Acid (ASA) in Mice and Hamsters
(U77-0240)

Testing Laboratory: [redacted]

Dates Started and Completed: October 4, 1976 and March 17, 1977

GLP and QAU Compliance Statement: None.

Methods: To examine the potential mutagenic effects of combination of persantine and ASA, micronucleus test was conducted using mouse and Chinese striped hamster bone marrow. Combination of persantine and ASA was given by oral gavage to mice or hamsters twice with a 24 hour interval at 0, 10, 100 and 1000 mg/kg. Rats were sacrificed 6 hours after the last dose and bone marrow was collected. Positive control (cyclophosphamide) was also tested. The frequency of micronucleated polychromatic erythrocytes was then determined.

Results: No treatment related clinical signs of toxicity were observed. Treatment with combination of persantine and ASA did not significantly increase the frequency of the micronucleated polychromatic erythrocytes compared to the control. The positive control, however, significantly increased the frequency of the micronucleated polychromatic erythrocytes compared to the control.

In conclusion, the results suggest that treatment with combination of persantine and ASA was not mutagenic in this test system.

Dominant Lethal Test with Combination of Persantine and Acetylsalicylic Acid (ASA) in Mice
(U77-0237)

Testing Laboratory: [redacted]

Dates Started and Completed: May 4, 1976 and January 10, 1977

GLP and QAU Compliance Statement: None.

Methods: The mutagenic effects of combination of persantine and ASA (1:4) were studied in mice using dominant lethal test. Male mice were given a single oral dose (gastric tube) of combination of persantine and ASA at 0, 10, 100 and 1000 mg/kg 24 hours before 2nd week of mating. The treated male mice were then mated with untreated females. The pregnant females were necropsied on day 14 of gestation and the following parameters were determined: numbers of corpora lutea, implantations (total, live, and dead), total and pre-implantation loss, live and dead fetuses. Positive control (cyclophosphamide, 100 mg/kg) was also tested.

Results: The results were presented in Table 10 on page 142 in volume 27 and this table is attached below.

PERSANTIN-ASA MÄUSE / MICE
WURFDATEN DER VERSCHIEDENEN PAARUNGSPERIODEN /
LITTER DATA OF DIFFERENT MATING PERIODS
MITTELWERTE / MEAN VALUES
(getötet 14. Tag / Sacrificed Day 14)

Paarungsperiode/ Mating period		1.	2.	3.	4.	5.	6.	7.	8.	9.
Kontrolle/ control	CL	13,2	13,1	14,5	14,1	13,3	13,1	12,3	13,3	12,7
	TI	12,2	12,7	13,3	13,0	13,1	12,7	12,3	12,2	12,6
	LI	11,2	11,7	12,3	12,0	11,9	12,1	11,8	11,6	12,1
	DI	1,1	1,0	1,1	1,0	1,2	0,7	0,5	0,6	0,5
	PIL	3,3	0,5	4,0	4,1	0,2	0,3	0	4,1	0,1
10 mg/kg	CL	13,6	13,4	13,7	13,7	13,1	13,9	12,2	12,9	13,3
	TI	12,7	11,8	12,4	11,3+	12,0	12,5	12,0	11,8	12,2
	LI	11,9	11,1	11,6	10,3	10,8	11,8	11,1	11,1	11,3
	DI	0,8	0,7	0,8	1,0	1,2	0,7+	0,9	0,7	0,9
	PIL	2,6	6,0	5,4	10,2	4,2	4,3+	0,2	3,8	4,3
100 mg/kg	CL	13,4	13,2	12,6+	13,7	13,5	13,4	12,1	13,0	12,6
	TI	12,3	11,8	12,0	11,7	12,1	12,0	11,9	11,4	12,0
	LI	11,2	11,3	11,4	10,6	11,1	11,3	11,3	10,8	11,4
	DI	1,1	0,5	0,6	1,0	0,9	0,8	0,5	0,5	0,5
	PIL	2,4	3,9	2,0	4,6	4,3	5,5+	0,3	5,5	0,8
1000 mg/kg	CL	13,5	14,3	13,0+	13,8	13,1	12,8	12,6	13,2	13,3
	TI	13,4	13,6	11,8	13,3	11,7	11,7	12,3	12,7	12,9
	LI	12,9	12,5	10,9	12,7	11,0	10,7	11,9	12,3	12,3
	DI	0,6	1,1	0,9	0,6	0,7	1,0	0,5	0,5	0,6
	PIL	0,1	0,9	3,4	1,5	4,6	2,4	0,3	1,2	0,7
100 mg/kg Cyclophos-phamid	CL	13,8	14,0	13,6	13,7	14,6	12,1	13,0	13,0	13,2
	TI	13,5	12,4	12,0+	12,6	13,7	11,7	12,0	12,4	11,9
	LI	12,7	9,6+	7,6+	10,5+	12,8	11,2	11,2	11,5	10,8
	DI	0,9	2,9+	4,4+	2,1+	0,9	0,5	0,8+	1,0	1,1
	PIL	0,3	6,9+	7,2	1,6	2,8	0,7	2,7+	1,7	2,4

CL: Corpora lutea / Muttertier
Corpora lutea / dam
TI: Gesamtimplantate / Muttertier
Total Implants / dam
LI: Lebende Implantate / Muttertier
Living Implants / dam
DI: Tote Implantate / Muttertier
Dead Implants / dam
PIL: Praeimplantationsverlust
Pre Implantation-Loss %

+ Diese Werte unterscheiden sich signifikant von den entsprechenden Kontrollwerten
These values differ significantly from the corresponding control values

The treatment with combination of persantine and ASA decreased number of corpora lutea in the mid (12.6/dam) and high (13/dam) dose groups and increased the preimplantation loss in the low (4.3%) and mid (5.5%) dose groups. The number of corpora lutea and preimplantation loss in the control group were 14.5/dam and 3.3%, respectively. The preimplantation loss in the high dose group (2.4%) was lower than those in the low and mid dose groups but higher than the control value (not statistically significant).

These changes may not be of any clinical significance. In addition, the positive control, cyclophosphamide, significantly decreased the number of live implantations/dam (7.6-9.6/dam) as compared to the control (11.7-12.3/dam). This was not seen in the treatment group with combination of persantine and ASA. In conclusion, it appears that the treatment with combination of persantine and ASA was not mutagenic in this test system.

LABELING:

The labeling is according to 21 CFR, Subpart B. The following revisions in the labeling are recommended.

1. Sponsor's Version:

Carcinogenesis, Mutagenesis, Impairment of Fertility:

Carcinogenesis: In carcinogenicity studies in rats and mice with the combination of dipyridamole and aspirin at the ratio of 1:6 over a period of 125 and 105 weeks respectively, no significant tumorigenic effect was observed at maximum doses of 450 mg/kg (corresponding to a share of 75 mg/kg of dipyridamole, 9 times the maximum recommended daily human dose for a 50 kg person on a mg/kg basis (or 1.5-2.1 times on a mg/m2 basis)), and 375 mg/kg aspirin, 375 times the maximum recommended daily human dose for a 50 kg person on a mg/kg basis (or 58-83 times on a mg/m2 basis).

Mutagenicity: In vivo and in vitro mutagenicity testing of the combination of dipyridamole and aspirin at a ratio of 1:5 revealed no signs to suggest a mutagenic risk.

Fertility: Fertility studies with dipyridamole revealed no evidence of impaired fertility in rats at oral dosages of up to 1250 mg/kg, 156 times the maximum recommended human dose on a mg/kg basis for a 50 kg person (or 35 times on a mg/m2 basis). Aspirin inhibits ovulation in rats.

Evaluation: The results of animal studies have not been fully reflected.

Suggested Version:

Carcinogenesis:

Dipyridamole: In a 111 week oral study in mice and in a 128-142 week oral study in rats, Persantine® (dipyridamole USP) produced no significant carcinogenic effects at doses of 8, 25 and 75 mg/kg. For a 50-kg person of average height (1.46 m^2 body surface area), the dose of dipyridamole at 75 mg/kg/day (225 mg/m^2/day in mice or 450 mg/m^2/day in rats) represents 0.76 or 1.5 times the recommended human dose (8 mg/kg/day or 296 mg/m^2/day) on a body surface area basis.

Combination of Dipyridamole and Aspirin: In a 105 week oral (in feed) study in mice and in a 125 week oral (in feed) study in rats, combination of dipyridamole and aspirin in a ratio of 1:5 produced no significant carcinogenic effects at doses of 50, 150, and 450 mg/kg/day. The dose of aspirin at 375 mg/kg/day (1125 mg/m^2 in mice or 2250 mg/m^2/day in rats) represents ~30 or 61 times the recommended human dose of aspirin in AGGRENOX (1 mg/kg/day or 37 mg/m^2/day) on a body surface area basis.

Mutagenicity:

Combination of Dipyridamole and Aspirin: Mutagenicity testing with combination of dipyridamole and aspirin in a ratio of 1:5 revealed no mutagenic potential in the Ames test, *in vivo* chromosome aberration tests in mice and hamsters, oral micronucleus tests in mice and hamsters and dominant lethal test in mice. Aspirin induced chromosome aberrations in cultured human fibroblasts.

Fertility:

Dipyridamole: Reproduction studies with Persantine revealed no evidence of impaired fertility in rats at oral dosages of up to 500 mg/kg/day or 3000 mg/m^2/day (~10 times the recommended human dose on a body surface area basis). A significant reduction in number of corpora lutea with consequent reduction in implantations and live fetuses was, however, observed at dose of Persantine of 1250 mg/kg/day or 7500 mg/m^2/day in rats (~25 times the recommended human dose on a body surface area basis).

Aspirin: Aspirin inhibits ovulation in rats.

Combination of Dipyridamole and Aspirin: Combination of dipyridamole and aspirin was not tested for effect on fertility and reproductive performance.

2. Sponsor's Version:

Pregnancy:

Pregnancy Category C: The combination of dipyridamole and acetylsalicylic acid at a ratio of 1:5.4 has been shown to exert embryotoxic effect in rabbits and rats when given at maternotoxic doses representing 3-9 times the maximum recommended human daily dose for dipyridamole for a 50 kg person on a mg/kg basis (or 1.3-2 times on a mg/m2 basis) and 110-330 times the maximum recommended human daily dose for aspirin for a 50 kg person on a mg/kg basis (or 47-73 times on a mg/m2 basis). There are no adequate and well-controlled studies of AGGRENOX in pregnant women. Because animal reproduction studies are not always predictive of human response, AGGRENOX should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Due to the aspirin component, AGGRENOX should be avoided in the third trimester of pregnancy.

Teratology studies have been performed in rats and rabbits using a dose ratio of dipyridamole:aspirin of 1:5.4. An increased resorption rate, which reached 100% in rats (75 mg/kg dipyridamole and 330 mg/kg aspirin) and at doses of 135 mg/kg in rabbits (25 mg/kg dipyridamole and 110 mg/kg aspirin). Malformations were observed exclusively in aspirin groups running concurrently but not in the dipyridamole/aspirin groups. Placental transfer of dipyridamole is very low.

Evaluation: The results of animal studies have not been fully reflected.

Suggested Version:

Dipyridamole: Pregnancy Category B: Reproduction studies with dipyridamole have been performed in mice at doses up to 125 mg/kg (375 mg/m^2, ~1.3 times the recommended human dose), in rats at doses up to 1000 mg/kg (6000 mg/m^2, 20 times the recommended human dose) and in rabbits at doses up to 40 mg/kg (480 mg/m^2, ~1.6 times the recommended human dose) and have revealed no evidence of harm to the fetus.

Aspirin: Pregnancy Category D: Aspirin may produce adverse maternal effects: anemia, ante- or postpartum hemorrhage, prolonged gestation and labor. Maternal aspirin use during later stages of pregnancy may cause adverse fetal effects: low birth weight, increased incidence of intracranial hemorrhage in premature infants, stillbiths, neonatal death. Aspirin should be avoided 1

week prior to and during labor and delivery because it can result in excessive blood loss at delivery.

Reproduction studies have been performed with combination of dipyridamole and aspirin in a ratio of 1:4.4 in rats and rabbits and have revealed no teratogenic evidence at doses of up to 405 mg/kg/day in rats and 135 mg/kg/day in rabbits. However, treatment with combination of dipyridamole and aspirin at 405 mg/kg/day induced abortion in rats. The doses of dipyridamole at 75 mg/kg/day represent 1.5 times the recommended human dose on a body surface area basis. In these studies, aspirin itself was teratogenic at doses of 330 mg/kg/day (1980 mg/m^2/day) in rats (spina bifida, exencephaly, microphthalmia, and coelosomia) and 110 mg/kg/day (1320 mg/m^2/day) in rabbits (congested fetuses, agenesis of skull and upper jaw, generalized edema with malformation of the head, and diaphanous skin). The doses of aspirin at 330 mg/kg/day in rats and at 110 mg/kg/day in rabbits were ~54 and 36 times the recommended human dose, respectively, on a body surface area basis.

There were no adequate and well-controlled studies in pregnant women. Aggrenox should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Due to the aspirin component, aggrenox should be avoided in the third trimester of pregnancy.

3. Sponsor's Version:

Overdose:

Evaluation: The results of the acute toxicity studies in animals are not included.

Suggested Version: A single oral dose of combination of dipyridamole and aspirin at doses of up to 6.75 g/kg in a ratio of 8:1 was non-lethal in rats. Decreased locomotor activity, prone position and piloerection were observed at doses of combination of dipyridamole and aspirin at 2.25 and 6.75 g/kg.

SUMMARY AND EVALUATION:

Aggrenox contains dipyridamole and aspirin at a ratio of 8:1. Dipyridamole is an antiplatelet agent. Its antiplatelet activity is related to its inhibition of adenosine uptake, inhibition of cGMP phosphodiesterase or increase in prostacyclin (PGI_2) level. By inhibition of adenosine uptake, it increases plasma level of adenosine which would activate the platelet A_2-receptor, stimulate platelet adenylate cyclase, and elevate platelet cAMP level. PGI_2 also activate platelet adenylate cyclase and increases cAMP level. The latter inhibits platelet activation. Inhibition of cGMP phosphodiesterease would increases cGMP level in the platelet, and like cAMP, cGMP can also inhibit platelet aggregation. Acetylsalicylic acid (ASA) irreversibly inhibits platelet cyclooxygenase and reduces the production of thromboxane A_2 (TXA_2). TXA_2 is a potent platelet aggregator. Since dipyridamole and aspirin inhibit platelet aggregation via independent mechanisms, use of both drugs in combination would produce an additive antiplatelet effect.

In the present NDA, sponsor is asking for approval to market AGGRENOX for reduction of the combined risk of death and nonfatal stroke in patients who have had transient ischemia of the brain or completed ischemic stroke. The recommended oral dose is one capsule twice daily. One capsule contains 200 mg dipyridamole and 25 mg aspirin (8:1). In support of this NDA, following preclinical studies of combination of dipyridamole and aspirin were submitted: pharmacological studies, pharmacokinetic study in dogs, acute oral/i.p. toxicity studies in mice and rats, acute oral toxicity study in dogs, 3-month oral toxicity study in rats, 24-week oral toxicity studies in rats and dogs, 27-week oral toxicity study in dogs, 102-week oral (in feeds) carcinogenicity study in mice, 125-week oral carcinogenicity study in rats, reproductive toxicity studies: Segment II oral teratological studies in rats and rabbits, mutagenicity studies: Ames test, *in vivo* chromosome aberration test in mice and hamsters, oral micronucleus test in mice and hamsters, and dominant lethal test in mice. All toxicity studies submitted in this submission are non-GLP studies conducted prior to the promulgation of the GLP regulations in 1979 and the ratio of dipyridamole to aspirin in these studies is not the same as that (8:1) in the drug product (AGGRENOX) except one acute oral toxicity study in rats (96B031). In the lack of required toxicity studies with right combination of dipyridamole and aspirin, sponsor

was asked to conduct 4-week oral toxicity studies in rats and dogs, and teratology studies in rats and rabbits with the drug combination of dipyridamole and aspirin in a ratio of 8:1 in the Division's letter dated October 28, 1997 in NDA 12,836. In response to this request, sponsor stated that they have conducted human pharmacokinetic interaction studies between dipyridamole and aspirin in a ratio of 8:1 and found no effect of dipyridamole on aspirin and vise versa in a telephone conference on November 24, 1997. The Division then concluded that the human data overrides the request to conduct toxicity studies. Therefore, no additional preclinical studies are required.

The human pharmacokinetic interaction study indicated that there is no pharmacokinetic interaction between dipyridamole and aspirin when given in the combination of dipyridamole (200 mg) and aspirin (25 mg) in a ratio of 8:1.

In the acute toxicity study with combination of dipyridamole and aspirin in a ratio of 8:1 in rats, decreased locomotor activity, prone position and piloerection was observed at doses of 2.25 and 6.75 g/kg and there were no deaths at doses up to 6.75 g/kg. In the acute toxicity studies with combination of dipyridamole and aspirin in a ratio of 1:4.5-5, central nervous system toxicities including sedation, prostration, and dyspnea were observed in both mice and rats. The minimal lethal dose was 1 g/kg (oral and i.p.) in mice. The minimal lethal dose in rats was 2 g/kg (oral) and 0.75 g/kg (i.p.) in rats. The minimal lethal oral dose in dogs was 0.937 g/kg. Sedation, lethargy and drowsiness were noted in dogs.

In the 3-month oral toxicity study in rats, the combination of persantine and aspirin (1:5) was given to rats at 0, 25, 100 and 400 mg/kg/day by oral gavage for 3 months. The only treatment related changes were decreased body weight gain in the high dose group (19-23%) as compared to the control. Based on the 19-23% reduction of body weight gain, it appears that the high dose of 400 mg/kg/day was slightly higher than MTD.

In the 24-week oral toxicity study in rats, the combination of persantine and aspirin (1:4) was given to rats orally at 0, 25, 100 and 400 mg/kg/day for 24 weeks. The treatment related changes were decreased body weight gain (~13%), prolongation of bleeding time (129%) and increase in urea (40%) and creatinine (36%) level in the high dose group. Histopathological examination revealed no treatment related changes. The high dose of 400 mg/kg/day was lethal.

In the 24-week oral toxicity study in dogs, the combination of persantine and aspirin (1:4) was given to dogs orally at 0, 25, 100, 200 and 400 mg/kg/day for 24 weeks. Dose of 400 mg/kg was extremely lethal and all animals died in this group. The major

treatment related changes were increase in urea and creatinine by 63-92% and 36-101% at 200 mg/kg, respectively. Creatinine was also slightly increased by 22-31% and 10-21% at 25 and 100 mg/kg at the end of treatment, respectively. Histopathological examination revealed nephritis and congestion in the liver, spleen, lung, kidney, and stomach mainly at 200 and 400 mg/kg.

In the 27-week oral toxicity study in dogs, dogs were treated orally with the combination of persantine and aspirin (1:5) at 0, 100 and 200 mg/kg/day and aspirin alone at 80 and 160 mg/kg/day for 27 weeks. The treatment with combination of persantine and aspirin produced cardiac toxicity as evidenced by increased heart rate and histopathological changes including ventricular hypertrophy and scar tissues in the ventricular papillary muscle. Pathological changes were also seen in the liver (degenerative fatty change in parenchyma of the liver), kidney (tubular atrophy and fat content in the epithelium of the proximal renal tubules), stomach (erosions of gastric mucosa), small intestine (hemorrhages in the jejunal mucosa and penetrating ulcer in the ileum), testes and epididymes (granulomatous arteritis and periarteritis). The target organs of toxicity were the heart, kidney, stomach, small intestinal, liver, testes and epididymes.

No carcinogenicity studies were conducted with combination of dipyridamole and aspirin in a ratio of 8:1. Persantine was negative in the 111-week oral carcinogenicity study in mice and 128-142 week oral carcinogenicity study in rats. Aspirin has been widely used in humans for many years and there was no indication of tumorigenicity. Therefore, there is no need to conduct 2-year carcinogenicity studies in animals.

In the Segment II teratologenic study in rats, combination of persantine and aspirin (1:4.4) was given orally at 0, 50.62, 202.5, and 405 mg/kg/day or aspirin alone at 330 mg/kg/day to pregnant female rats from gestation days 6 to 15. Maternal toxicity was noted in the treatment groups as evidenced by decreased body weight (14%, 29%, 43% and 47% in the low, mid and high dose groups and aspirin alone group, respectively). Treatment with combination of persantine and aspirin induced abortion (1/18 in low dose group and 17/18 in high dose group) and decreased fetus weight in the mid (12%) and high (68%) dose groups. No teratogenic evidence was found in the animals treated with the combination of persantine and aspirin. Treatment with aspirin produced a number of major malformations (spina bifida, exencephaly, microphthalmia, and coelosomia) and thus was teratologic.

In the Segment II teratologenic study in rabbits, pregnant rabbits were treated orally with combination of persantine and aspirin (1:4.4) at 0, 27, 81, and 135 mg/kg/day or aspirin alone at 110 mg/kg/day to pregnant female rabbits from gestation days 6 to 18. Reduction of body weight gain was noted in the high dose

group. In the aspirin group, following external anomalies were noted: congested fetuses, agenesis of skull and upper jaw, generalized edema with malformation of the head, and diaphanous skin. In conclusion, aspirin was teratologic in this study.

Malformations associated with aspirin were also reported previously in animals. These included cleft lip, execephaly, microcephaly, and spina bifida in mice (Trasler, 1965, Lancet, 1:606), hydrocephalus, and cleft palate in rats (Kimmel, et al., 1971, Teratology, 4:15, McColl et al., 1965, Toxicol. Appl. Pharmacol. 7:409), heart and rib defects in rabbits (McColl et al., 1967, Toxicol. Appl. Pharmacol., 10:244), cleft palate, micrognathia, anasarca, cardiovascular malformations and tail anomalies in dogs (Robertson et al., 1979, Teratology, 20:31), and heart defects in rhesus monkeys (Wilson, 1971, Fed. Proc., 30:104).

The treatment with combination of persantine and ASA (1:5) was not mutagenic in the Ames test, *in vivo* chromosome aberration tests in mice and hamsters, oral micronucleus tests in mice and hamsters and dominant lethal test in mice.

In summary, aggrenox contains dipyridamole and aspirin in a ratio of 8:1. Both dipyridamole (persantine) and aspirin are approved drugs and have been used in humans for many years. The pharmacological studies demonstrated that use of dipyridamole and aspirin in combination produces an additive antiplatelet effect since these two drugs inhibit platelet aggregation via independent mechanisms. The human pharmacokinetic interaction study indicated that there is no pharmacokinetic interaction between dipyridamole and aspirin when given in the combination of dipyridamole and aspirin in a ratio of 8:1. The human experience with aspirin suggests that aspirin is not tumorigenic. Based on the available information, it appears that the combination use of dipyridamole and aspirin may not produce more toxicity or risk to humans than use of each compound alone. Therefore, from a preclinical standpoint, this NDA is approvable. Sponsor should be asked to revise the labeling as recommended.

RECOMMENDATION:

From a preclinical standpoint, this NDA is approvable. Sponsor should be asked to revise the labeling as recommended.

/S/ 4/30/99

Ke Zhang, Ph.D.

/S/ 4/30/99

ATTACHMENT: Appendix I

Tumor Data for 105 Week Carcinogenitity Study in Mice and
125 Week Carcinogenicity Study in Rats
(Pages 50 - 53)

cc:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang
HFD-345/Dr. Viswanathan

R/D Init.: J. Choudary 4/9/99

KZ/hw/4/21/99 & 4/23/99
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Appendix I

Tumor Data for 105 Week Carcinogenicity Study in Mice
(Page 51)

And

125 Week Carcinogenicity Study in Rats
(Page 52 - 53)

APPEARS THIS WAY
ON ORIGINAL

TABLE 10

Type of tumours/localisation		Group (I) Control		Group (II) 50 mg P + A/kg		Group (III) 150 mg P + A/kg		Group (IV) 450 mg P + A/kg	
		m	f	m	f	m	f	m	f
Benign:									
Adenoma: Lung		4	7	6	1	3	0	1	0
Pituitary		0	1	0	0	0	1	0	0
Salivary gland		1	0	0	0	0	0	0	0
Adrenal		1	0	0	0	0	0	0	0
Thyroid gland		0	0	0	0	0	1	0	0
Haemangio-endothelioma	Liver	2	0	2	1	5	1	2	0
	Spleen	2	0	0	1	1	0	1	0
	Kidney	0	0	0	0	1	0	0	0
Haemangioma	Liver	1	0	1	0	0	1	0	0
	Spleen	0	1	0	0	0	0	0	0
	Uterus	-	0	-	0	-	0	-	1
Hepatoma:	Liver	0	0	1	1	0	2	0	0
Nodular hyperplasia:	Liver	0	0	0	0	1	0	0	0
Interstitial cell tumour:	Testis	2	-	0	-	0	-	0	-
Granulosa-cell tumour:	Ovary	-	6	-	0	-	2	-	1
Corpus polyp:	Uterus	-	1	-	0	-	2	-	0
Myoma:	Uterus	-	2	-	2	-	0	-	5
Total of benign tumours		13	18	9	6	11	10	4	7
Malignant:									
Leukaemia: Reticuloendothelial system/blood		4	6	2	4	1	5	0	1
Lymphoma: Reticuloendothelial system/blood		14	24	14	13	4	11	10	11
Adenocarcinoma:	Lung	0	1	0	0	0	0	1	0
	Uterus	-	0	-	1	-	1	-	0
	Pelvis	0	0	0	0	0	0	1	0
malignant hepatoma:	Liver	1	1	0	0	0	0	0	0
Phaeochromocytoma	Kidney	0	0	0	0	1	0	0	0
Sertoli cell tumour	Testis	1	-	0	-	0	-	2	-
Sarcoma: Uterus		-	1	-	1	-	4	-	0
Bone		0	0	0	1	0	0	0	0
Total of malignant tumours		20	33	16	20	6	21	14	12

TABLE 11 Comparison of benign/malignant tumours in rats

Nature of tumours/ localisation	Group (I) control		(II) P + A dose in mg/kg B.W. in the food 50		(III) 150		(IV) 450	
	m	f	m	f	m	f	m	f
Benign								
Cavernoma: spleen	1	0	0	0	0	0	0	0
Hepatoma: liver	1	0	2	0	0	1	1	0
haemangioma: liver	0	0	1	0	0	0	0	0
ovary	-	1	-	0	-	0	-	0
lymph nodes	0	0	0	0	1	0	0	0
Nodular hyperplasia: liver	0	0	1	0	0	0	0	0
Adenoma: lung	1	0	1	0	0	0	1	1
thyroid	4	5	4	10	4	5	1	5
parathyroid	4	0	0	0	0	0	0	1
adrenal	1	0	0	1	0	0	0	0
pituitary	11	43	3	13	5	16	4	18
Insuloma: pancreas	1	2	1	0	1	0	1	1
Teratoma: pancreas	1	0	0	0	0	0	0	0
Neurinoma: nerve	0	0	0	1	0	0	0	0
Haemangioendothelioma:								
lymph nodes	0	0	1	0	0	0	0	0
thymus	0	0	0	0	0	1	0	0
Interstitial cell tumour: testes	45	-	15	-	20	-	15	-
Dermoid cysts: ovary	-	1	-	0	-	0	-	0
Granulosa cell tumour: ovary	-	3	-	4	-	0	-	1
Thecoma: ovary	-	2	-	0	-	0	-	0
Luteoma: ovary	-	0	-	1	-	0	-	0
Cophoroma: ovary	-	0	-	0	-	1	-	0
Myomatosis: uterus	-	1	-	0	-	0	-	0
Corpus polyp: uterus	-	0	-	0	-	0	-	1
Myoma: uterus	-	1	-	0	-	0	-	0
abdomen	0	1	0	0	0	0	0	0
Adenofibroma: mammary gland	0	0	0	0	0	0	0	0
Fibro-adenoma: mammary gland	1	3	0	4	0	0	0	4
neck	0	0	0	0	0	1	0	0
abdomen	0	0	0	1	0	0	0	0
Fibromatosis: abdomen	1	0	0	0	0	0	0	0
extremity	1	0	0	0	0	0	0	0
Fibroma: abdomen	0	0	0	1	0	1	0	0
skin	0	0	0	0	0	1	0	0
Papilloma: abdomen	1	0	0	0	0	0	0	0
bladder	1	0	0	0	0	0	0	0
Papillomatosis: bladder	1	0	0	0	2	0	0	0
Haemangiopericytoma: head	1	0	0	0	0	0	0	0
extremity	1	0	0	0	0	0	0	0
spleen	0	0	0	0	0	0	1	0
Lipoma: extremity	0	0	0	0	1	0	0	0
Cystadenoma: salivary gland	0	1	0	0	0	0	0	0
Total of benign tumours	78	64	32	36	34	29	24	36

TABLE 11 Comparison of benign/malignant tumours in rats

Nature of tumours/ localisation	Group (I) control		(II) P + A dose in mg/kg B.W. in the food 50		(III) 150		(IV) 450	
	m	f	m	f	m	f	m	f
Malignant								
Sarcoma: kidney	0	0	0	0	0	0	0	1
lung	0	0	0	0	0	1	0	0
uterus	-	1	0	0	0	0	0	0
abdomen	1	0	0	0	0	0	0	0
Myosarcoma: uterus	-	0	-	0	-	0	-	1
abdomen	0	0	0	1	0	0	0	0
Histiosarcoma: abdomen	0	1	0	0	0	0	0	0
Fibrosarcoma: lung	0	1	0	0	0	0	0	0
extremity	6	2	0	0	0	0	0	0
head	1	0	1	0	0	1	0	0
abdomen	1	1	0	1	1	0	0	0
lymph nodes	0	0	0	1	0	0	0	0
Spindle-cell sarcoma: extremity	0	0	0	0	0	0	0	1
Liposarcoma: abdomen	0	0	0	0	0	1	0	0
pelvis	0	0	0	0	0	0	0	1
Carcinoma: pelvis	1	0	0	0	0	0	0	0
Adenocarcinoma: uterus	-	2	-	1	-	1	-	0
mammary gland	0	0	0	0	0	0	0	1
small pelvis	0	0	1	0	0	0	0	0
Squamous cell carcinoma:								
uterus	-	1	-	0	-	0	-	1
bladder	1	0	0	0	0	0	0	0
abdomen	0	0	0	1	0	0	0	0
head	1	0	0	0	0	0	0	0
Carcinoma solidum: head	0	1	0	0	0	0	0	0
Reticulosarcoma: spleen	0	0	-2	0	0	0	0	0
Phaeochromocytoma adrenal	5	5	8	0	0	4	1	1
Glioblastoma: head	2	1	0	0	0	0	0	0
Thymoma: thymus	1	2	4	4	4	7	2	3
lymphoma: reticulo-endothelial system/blood	5	3	1	0	1	0	2	0
Leukaemia: reticulo-endothelial system/blood	1	1	1	0	0	0	2	2
Reticulosis: reticulo-endothelial system/blood	0	1	0	1	0	0	0	2
Necrotic tumour*: head	0	0	0	0	1?	0	0	0
Total of malignant tumours	26	23	18	10	7?	15	7	14

* Probably malignant, but this could not be firmly established.

DuBeau

MEMORANDUM DEPARTMENT OF HEALTH AND HUMAN SERVICE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: May 21, 1999

MAY 30 1999

FROM: Ke Zhang, Ph.D.
Pharmacologist, HFD-180

SUBJECT: Statistical Analysis of Incidence of Thymoma in the Rat Carcinogenicity Study in NDA 20,884

TO: NDA 20,884

In the Executive CAC meeting held on May 11, 1999, one of the recommendations from the Committee was to conduct a statistical analysis on the incidence of thymoma in the 125-week oral carcinogenicity study in rats submitted in NDA 20,884. The mortality and the tumor incidence in this study are summarized in the following tables.

Mortality and incidence of thymoma in the paranthesis in male rats

Week	Control	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
0-52	2 (0)	2 (0)	1 (0)	0 (0)
53-78	6 (1)	1 (0)	3 (0)	4 (0)
79-92	2 (0)	6 (1)	4 (0)	5 (0)
93-104	11 (0)	6 (0)	7 (0)	2 (0)
105-124	33 (0)	17 (2)	20 (0)	15 (0)
Terminal sacrifice	46 (0)	18 (1)	15 (4)	24 (2)
Total incidence of thymoma	1	4	4	2

Mortality and incidence of thymoma in the paranthesis in female rats

Week	Control	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
0-52	1 (0)	1 (0)	0 (0)	1 (0)
53-78	5 (0)	4 (0)	0 (0)	3 (0)
79-92	11 (0)	4 (0)	1 (0)	5 (0)
93-104	8 (0)	5 (0)	9 (0)	3 (0)
105-124	28 (0)	12 (1)	14 (1)	17 (1)
Terminal sacrifice	47 (2)	24 (3)	26 (6)	22 (2)
Total incidence of thymoma	2	4	7	3

Dr. Karl Lin (CDER statistician) has conducted the mortality adjusted exact permutation trend and pairwise tests on this tumor incidence (see attachment). The results indicated that there was no statistical significance for both males and females in both pairwise and trend tests. The P values (trend test) are 0.398 for males and 0.215 for females. The P values of the pairwise test are summarized in the following table.

	50 mg/kg/day	150 mg/kg/day	450 mg/kg/day
Males	0.0469	0.016	0.297
Females	0.092	0.333	0.3778

The positive trend is tested at 0.025 and 0.005 levels of significance for rare and common tumors, respectively. For pairwise comparison tests the levels of significance are 0.05 and 0.01 for rare and common tumors, respectively. The tumor incidence was counted in 100 animals per sex in the control group and 50 animals per sex in each of the treatment groups.

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/S/ 5/21/99
Ke Zhang, Ph.D.

Attachment: Dr. Karl Lin's summary of the statistical analysis.

/S/ 5/30/99

cc:
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang
HFD-715/Dr. Lin

R/D Init.: J. Choudary 5/18/99

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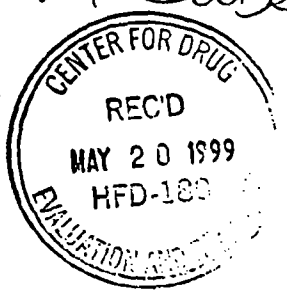

Executive CAC
May 11, 1999

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
Paul Andrews, Ph.D., HFD-150, Alternate Member
Jasti Choudary, B.V.Sc., Ph.D., Team Leader
Ke Zhang, Ph.D., Presenting Reviewer

Author of Draft: Ke Zhang, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA #: 20,884
Drug Name: AGGRENOX (dipyridamole and aspirin)
Sponsor: Boehriger Ingelheim

Background: AGGRENOX is a combined drug product containing 200 mg dipyridamole and 25 mg aspirin (8:1). AGGRENOX is indicated for reduction of the combined risk of death and nonfatal stroke in patients who have had transient ischemia of the brain or completed ischemic stroke. No carcinogenicity studies were conducted with drug combination of dipyridamole and aspirin in a ratio of 8:1 as in AGGRENOX. However, a 105-week oral (in feed) carcinogenicity study in mice and a 125-week oral (in feed) carcinogenicity study in rats were conducted using the drug combination of dipyridamole and aspirin in a ratio of 1:5. These studies were conducted prior to the promulgation of GLP regulations. Sponsor did not provide the basis of dose selection. Both dipyridamole (persantine) and aspirin are approved drug products. Persantine was not carcinogenic in the 111-week oral carcinogenicity study in mice and 128-142 week oral carcinogenicity study in rats. Aspirin has been widely used in humans for many years and there was no indication of tumorigenicity.

Mouse Carcinogenicity Study:

In the 105-week oral carcinogenicity study in mice, combination of dipyridamole and aspirin (1:5) was given to mice in feed at 0, 50, 150 and 450 mg/kg/day for 105 weeks. There were no treatment related changes in body weight and histopathology. The mortality and tumor rates were comparable in all groups.

Rat Carcinogenicity Studies:

In the 125-week oral carcinogenicity study in rats, combination of dipyridamole and aspirin (1:5) was given to rats in feed at 0, 50, 150 and 450 mg/kg/day for 125 weeks. The terminal body weight was lower in the high dose females (85% of the

control) and males (95% of the control). Body weights were decreased in the high dose group over the full course of the treatment with exception of study termination. The mortality was comparable in all groups. A total of 100 animals per sex in the control group and 50 animals per sex in each of the treatment groups were examined histopathologically.

Executive CAC Recommendations and Conclusions:

1. In the mouse study, it was not clear whether MTD was reached at the doses tested.

2. In the rat study, the high dose of 450 mg/kg/day is considered as MTD based on the body weight changes during the study.

3. Sponsor should be asked to clarify how the conduct of the mouse and rat studies deviated from GLP regulations and the significance of these deviations. The results of these studies could be used in the labeling if the deviations are acceptable.

4. The Committee recommended to have the statisticians do a test to determine the significance of the incidence of the thymoma in the rat study.

5. The Committee also recommended to obtain available data on aspirin plasma clearance in mice versus humans from literature to aide in determining the relevance of the different ratio of drugs present in the tested versus the to be marketed formulation.

6. The above requested information should be transmitted to the CAC Executive Committee via e-mail.

/S/ 5/24/99

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

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/Division File, HFD-180
/HFD-181/CSO
/Dr. Choudary, HFD-180
/Dr. Zhang, HFD-180
/ASeifried, HFD-024

DuBeau

M E M O R A N D U M DEPARTMENT OF HEALTH AND HUMAN SERVICE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: May 13, 1999

FROM: Ke Zhang, Ph.D.
Pharmacologist, HFD-180

SUBJECT: Recommendation of the Executive CAC Committee on the carcinogenicity studies in mice and rats in NDA 20,884

TO: NDA 20,884

In NDA 20,884, sponsor (Boehringer Ingelheim) submitted the 105-week oral (in feed) carcinogenicity study in mice and 125-week oral (in feed) carcinogenicity study in rats with drug combination of dipyridamole and aspirin in a ratio of 1:5. These studies were conducted prior to promulgation of GLP regulations. The Executive CAC Committee discussed the review of these studies on May 11, 1999 and made some suggestions. For further review of these studies, the following information is needed:

1. Sponsor should be asked to clarify how the conduct of the mouse and rat carcinogenicity studies (U79-0257 and U79-0258) deviated from GLP regulations and the significance of these deviations.

2. Statistical analysis on the incidence of thymoma in the 125-week oral carcinogenicity study in rats should be conducted by both sponsor and FDA to determine the significance of this tumor (both trend test and pairwise test). Dr. Karl Lin has done a preliminary analysis.

3. Sponsor should also be asked to provide the historical control data of the tumor incidence in Chbb:THOM rats in the testing laboratory during 1974-1979.

Sponsor should be asked to provide the above requested information as soon as possible.

/S/ 5/13/99
Ke Zhang, Ph.D.

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HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang

R/D Init.: J. Choudary 5/12/99

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